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THEMATIC LIST OF SESSIONS

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

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

Session Number	Session Title	Type	Day & Time Mon.	Tue.	Wed.	Thu.	Fri.
108.	Comparative neuroanatomy: reptiles, birds, mammals	Poster	mPM				
546.	Hippocampus and amygdala: behavior	Poster					fAM
53.	Hippocampus and amygdala: neuroanatomy	Poster	mAM				
181.	Hippocampus and amygdala: neurocytology	Poster		tuAM			
306.	Hippocampus and amygdala: neurophysiology I	Poster			wAM		
448.	Hippocampus and amygdala: neurophysiology II	Poster				thAM	
240.	Hypothalamus	Poster		tuPM			
54.	Limbic system I	Poster	mAM				
	Theme I: Neural Basis of Behavior						
248.	Biological rhythms and sleep I	Poster		tuPM			
270.	Biological rhythms and sleep II	Slide			wAM		
317.	Biological rhythms and sleep III	Poster			wAM		
514.	Biological rhythms and sleep IV	Poster				thPM	
550.	Biological rhythms and sleep V	Poster					fAM
451.	Drugs of abuse	Poster				thAM	
57.	Drugs of abuse: alcohol I	Poster	mAM				
112.	Drugs of abuse: alcohol II	Poster	mPM				
183.	Drugs of abuse: alcohol III	Poster		tuAM			
198.	Drugs of abuse: alcohol IV	Slide		tuPM			
312.	Drugs of abuse: alcohol V	Poster			wAM		
243.	Drugs of abuse: amphetamine	Poster		tuPM			
136.	Drugs of abuse: amphetamine and cocaine	Slide		tuAM			
450.	Drugs of abuse: cannabinoids, nicotine and PCP	Poster				thAM	
11.	Drugs of abuse: cocaine	Slide	mAM				
242.	Drugs of abuse: cocaine and others	Poster		tuPM			
310.	Drugs of abuse: cocaine, behavior	Poster			wAM		
309.	Drugs of abuse: cocaine, cellular	Poster			wAM		
110.	Drugs of abuse: cocaine, dopamine	Poster	mPM				
111.	Drugs of abuse: cocaine, serotonin	Poster	mPM				
382.	Drugs of abuse: opioids	Poster			wPM		
204.	Hormonal control of behavior I	Slide		tuPM			
308.	Hormonal control of behavior II	Poster			wAM		
315.	Hormonal control of behavior III	Poster			wAM		
380.	Hormonal control of behavior IV	Poster			wPM		
18.	Human behavioral neurobiology	Slide	mAM				
241.	Human behavioral neurobiology: event related potentials, attention, audition	Poster		tuPM			
509.	Human behavioral neurobiology: history, memory, imaging, other	Poster				thPM	
513.	Ingestive behavior: body weight and eating	Poster				thPM	
376.	Ingestive behavior: monoamines	Poster			wPM		
131.	Ingestive behavior: monoamines and nutrients	Slide		tuAM			
318.	Ingestive behavior: peptides I	Poster			wAM		
406.	Ingestive behavior: peptides II	Slide				thAM	
510.	Ingestive behavior: salt, water and aversion	Poster				thPM	
381.	Interhemispheric relations	Poster			wPM		
14.	Invertebrate learning and behavior I	Slide	mAM				
246.	Invertebrate learning and behavior II	Poster		tuPM			
263.	Invertebrate learning and behavior III	Slide			wAM		
249.	Learning and memory: anatomy I	Poster		tuPM			
250.	Learning and memory: anatomy II	Poster		tuPM			
115.	Learning and memory: conditioning	Poster	mPM				
127.	Learning and memory: human lesion studies	Slide		tuAM			
258.	Learning and memory: non-human primate lesion studies	Slide			wAM		
549.	Learning and memory—pharmacology	Poster					fAM
58.	Learning and memory—pharmacology: acetylcholine I	Poster	mAM				
374.	Learning and memory—pharmacology: acetylcholine II	Poster			wPM		
316.	Learning and memory—pharmacology: excitatory amino acids	Poster			wAM		
508.	Learning and memory—pharmacology: monoamines	Poster				thPM	
113.	Learning and memory: physiology I	Poster	mPM				
185.	Learning and memory: physiology II	Poster		tuAM			
205.	Learning and memory: physiology III	Slide		tuPM			
314.	Learning and memory: physiology IV	Poster			wAM		

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

Theme J: Disorders of the Nervous System

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

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

Other:

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

374.13

SCOPOLAMINE DISRUPTS WORKING MEMORY MORE IN THE MORNING THAN IN THE AFTERNOON. S.D. Drastal*, W.N. Tapp, B.H. Natelson, T.A. Pritzel*. VA Medical Center & New Jersey Medical School, East Orange, NJ 07019.

Performance fluctuates across the day in both people and animals. To examine the psychobiological influences on performance rhythms, this study determined the role of working memory in daily performance of rhesus monkeys. We studied the effects of scopolamine on performance at two different times of the day. Scopolamine injections were given at 1 and 5 hrs after lights-on. We used doses reported to affect working memory selectively.

To earn their food, monkeys had to perform a vigilance task followed by a color-position discrimination task. Trials were delivered on the average of every 2.4 min, and response latencies and choices were recorded. We noticed there was a pause in performance after the scopolamine injection. This pause lasted longer after the morning dose than in the afternoon and their performance recovered sooner in the afternoon.

Normally, these monkeys made only 1 to 3 discrimination mistakes per day. We compared performance for the 5 hrs following scopolamine with the comparable time period in baseline nodrug- and saline-injected conditions. The monkeys made 5.15 times more mistakes when given scopolamine ($p < 0.001$) . The monkeys made nearly twice the number of mistakes in the morning (6.78) as they did in the afternoon (3.52) following a scopolamine injection ($p < 0.02$).

The time of day differences in mistakes under the influence of scopolamine suggests that working memory is more susceptible to disruption in the morning. This pattern of susceptibility might be adaptive if we assume that natural disruptions of memory, such as fatigue, are more likely to occur in the afternoon when the animal is resistant to such disruptions. Supported by VA Research.

374.14

CONTINUOUS INTRACEREBROVENTRICULAR (ICV) INFUSION OF SCOPOLAMINE IMPAIRS CONTINUOUS PERFORMANCE IN RHESUS MONKEYS. M.J. Callahan, J. Kinsora, R.E. Davis, R. Harbaugh and T. Reeder. Parke-Davis Pharm. Res. Div., Warner-Lambert Co., Ann Arbor, MI 48105 and Dept. of Neurosurgery, Dartmouth Med. Sch., Hanover, NH 03756.

Scopolamine impairs cognitive function in animals and humans when adminstered systemically. Since scopolamine has profound effects on peripheral and central cholinergic function, we developed a convenient refillable system for continuously adminstering compounds to the central nervous system of rhesus monkeys.

Two eight year old male rhesus monkeys were implanted with a subcutaneous infusion pump connected to a cannulae stereotaxically directed toward the right lateral ventricle. Continuous ICV infusion of scopolamine produced a dose-dependent decrease in the number of responses. The magnitude of the response decrement produced by scopolamine was dependent on the stimulus duration with fewer responses occurring at the shorter stimulus durations. This decrease in performance was also time dependent and occurred mainly during the last half of the test session.

These data suggest that scopolamine produces a deficit in sustained attention that is mediated through direct central cholinergic blockade in the rhesus monkey. These procedures were well tolerated and the system has remained functional over an extended time period.

374.15

EFFECTS OF SCOPOLAMINE AND CLONIDINE ON RECOGNITION MEMORY IN RHESUS MONKEYS WITH LESIONS OF THE BASAL FOREBRAIN CHOLINERGIC SYSTEM. C. Chavoix, T. Aigner, H. Ogura* and M. Mishkin. Laboratory of Neuropsychology, NIMH, Bethesda, MD, 20892.

Both acetylcholine (ACh) and norepinephrine (NE) are thought to play critical roles in processes of memory. Recent evidence suggests that NE may influence the activity of ACh neurons in the basal forebrain (BF). In an attempt to investigate the mnemonic contributions of these 2 neurotransmitters, we administered the muscarinic antagonist scopolamine (SCOP) and the α_2 agonist clonidine (CLON) to 4 monkeys with BF lesions (group L) and 3 unoperated control monkeys (group C). Animals were first trained in delayed nonmatching-to-sample with a 10-s delay between sample presentation and choice. Bilateral BF lesions were then made by injecting ibotenic acid into 17 target sites in each hemisphere. Magnetic resonance imaging was used to locate the target sites and to confirm the accuracy of the lesions. Postoperatively, group L was slower than group C to relearn the task, but performed normally at delays up to 120 s. At the most difficult test of remembering a list of 20 items, performance of group C improved by 14% with continued practice, whereas that of group L remained the same. Although SCOP (0.32, 1, 3.2, 5.6, 10 & 17.8 µg/kg, IM) impaired the performance of both groups, the effect was greater in group L. Moreover, CLON (0.032, 0.1, 0.32, 1, 3.2, 10, 32, and 42.3 µg/kg, IM) differentially affected performance in the two groups. These results emphasize the role of ACh and, further, suggest a complementary role of NE in recognition memory in primates.

374.16

SINGLE DOSE PHARMACO-EEG PREDICTS RESPONSE TO TETRAHYDROAMINOACRIDINE TREATMENT IN ALZHEIMER'S DISEASE. H. Soininen*, K. Alhainen*, J. Partanen* and P. Riekkinen. Dept. of Neurology and Clinical Neurophysiology, University of Kuopio, P.O.Box 6, 70211 Kuopio, Finland.

Only 30-50% of Alzheimer (AD) patients benefit from various cholinomimetics such as tetrahydroaminoacridine (THA). Because we recently showed that progressive EEG decline is perhaps related to cell damage of the nucleus basalis of Meynert, it was reasonable to assume that EEG may help to identify responders to THA. We selected 12 AD patients (NINCDS-ADRDA criteria) and 7 controls with AAMI (age associated memory impairment without dementia). AD patients had 7-week THA treatment and seven of them were responders. In both groups a baseline EEG and another EEG at 90 min after a single dose of 50 mg THA were recorded.

In responders alpha-delta ratio increased 57.6% and alpha-theta ratio increased 61.4% compared to baseline. In nonresponders alpha-delta ratio and alpha-theta ratio decreased 33.3% and 10.7%, respectively. The AAMI group showed no change in these parameters. These results suggest that a single dose pharmaco-EEG predicts treatment response to THA.

NEUROPEPTIDES AND BEHAVIOR III

375.1

MU-OPIOID AGONIST AUGMENTS LOCOMOTOR ACTIVITY AFTER BILATERAL 6-HYDROXYDOPAMINE LESIONS IN THE NUCLEUS ACCUMBENS WITHOUT INVOLVING RECEPTOR UPREGULATION. L. Churchill and P.W. Kalivas. Dept. of VCAPP, Washington State University, Pullman, WA 99164-6520.

The mesolimbic dopamine system was lesioned by bilateral injection of 6-hydroxydopamine into the nucleus accumbens of rats. Ten days or more after the lesion, loçomotor activity increased to a significantly greater extent after microinjection of the μ-opioid agonist, Tyr-D-Ala-Gly-mePhe-Gly-OH (DAGO). A much smaller lesion-induced increase was observed after the δ-opioid agonist [D-penicillamine^{2,5}]-enkephalin (DPDPE). Receptor autoradiography with [^{125}I]DAGO or [^{125}I]DPDPE binding to the opioid receptors showed no significant differences between control and lesioned rats at 10 days after the lesion. Forskolin (1 μM) stimulated adenylate cyclase to a greater extent in the lesioned rats than in the sham treated rats (ascorbic acid treatment), but the opioid inhibition of forskolin stimulation did not differ between controls and lesioned rats. DAGO appeared more effective than DPDPE at inhibiting forskolin-stimulated adenylate cyclase. In conclusion, the behavioral analyses argue for a greater upregulation of the μ-opioid induced motor activity than that of the δ-opioid after dopamine depletion. The mechanisms underlying this augmentation do not appear to be caused by an increase in opioid receptor binding or inhibition of adenylate cyclase.

375.2

NEUROENDOCRINE MODULATION OF LOCOMOTOR ACTIVITY IN AN AMPHIBIAN: CORTICOTROPIN-RELEASING FACTOR AND OPIOIDS. C.A. Lowry, P. Deviche and F.L. Moore. Oregon State Univ., Corvallis, OR 97331.

Studies using mammals indicate that corticotropin-releasing factor (CRF) may coordinate physiological, autonomic and behavioral responses to stressful stimuli. We present evidence that CRF is involved in regulating stress-induced behavior in amphibians and further that the behavioral effects of CRF are modulated by the opioid system. Intracerebroventricular (icv) injections of ovine CRF dose-dependently stimulated locomotor activity in male rough-skinned newts (*Taricha granulosa*). This effect of CRF was completely blocked by the CRF receptor antagonist, α-helical CRF$_{9\text{-}41}$ (ahCRF). In other experiments, exposure to stressful environmental stimuli (either warm water or a 30 s period of handling) also stimulated locomotor activity. Injections of ahCRF reduced stress-induced locomotor activity regardless of the nature of the stressful stimulus.

Studies with opioid agonists and antagonists suggest that CRF and opioids interact to control locomotor activity in *T. granulosa*. The opioid μ-receptor agonist, morphine, at a dose that did not affect spontaneous locomotor activity, suppressed CRF-induced locomotor activity in a naloxone-reversible manner. In contrast, the preferential opioid kappa-receptor agonist, bremazocine, which dose-dependently suppressed spontaneous locomotor activity, did not affect CRF-induced locomotor activity. Experiments using the opioid receptor antagonist, naloxone, found that naloxone had no effect on spontaneous locomotor activity but under certain conditions enhanced CRF-induced locomotor activity. Although the effect of naloxone on CRF-induced locomotor activity is opposite to that typically observed in mammals, our other results are consistent with observations in mammals. Thus the neuroendocrine mechanisms controlling stress-induced locomotor activity appear to be evolutionarily conserved among vertebrates.

375.3

PERIPHERALLY ADMINISTERED SUBSTANCE P: EFFECTS ON FUNCTIONAL RECOVERY AFTER NIGROSTRIATAL 6-OHDA LESIONS AND ON STRIATAL DOPAMINE AS MEASURED BY IN-VIVO MICRODIALYSIS.
F.Boix*, R.Mattioli*, F.Adams*, R.K.W.Schwarting and J.P. Huston. Inst. of Physiol. Psychol. I, Univ. of Düsseldorf, Universitätsstr. 1, 4000 Düsseldorf, FRG

A close neural and functional relationship has been shown between nigrostriatal dopamine (DA) and the neuropeptide substance P (SP). Furthermore, SP can improve recovery after lesions of neural tissue. These results indicate that SP might have ameliorating effects after lesions aimed at nigrostriatal DA. To test this hypothesis, rats received daily injections of SP (50ug/kg, ip) after unilateral 6-OHDA lesions of the substantia nigra. 12 hours after each injection, they were tested for behavioral asymmetries. SP affected behavior in animals with moderate DA depletions. These animals showed no asymmetry in turning and an enhanced recovery from the asymmetry in scanning behavior. They also showed an increased striatal DA metabolism (post mortem). In a second study, the effect of SP was analyzed on extracellular DA in intact, freely-moving animals by means of the microdialysis technique. SP increased neostriatal DA, and this effect could be observed for several hours. These results demonstrate behavioral and neurochemical effects of SP related to DAergic mechanisms both in intact and brain-damaged animals. They are discussed with respect to the possible central and peripheral actions by which this neuropeptide may affect central DA and the recovery from DAergic lesions.

375.4

CHOLECYSTOKININ-INDUCED REDUCTION IN BODY TEMPERATURE ABOLISHED BY VENTRAL TRUNK VAGOTOMY.
E.H. South and P.D. Huff*, Dept.of Vet.Sci., Univ.of Idaho,Moscow,ID 83843.

The sensory neurotoxin, capsaicin, abolishes reduction of body temperature as well as the vagally mediated suppression of food intake by cholecystokinin octapeptide (CCK)(South,et al;NS Abst,1989). We now find that rats with a complete transection of the subdiaphragmatic ventral trunk of the vagus (VAGX) do not reduce rectal temperature (RT) in response to 4 µg/kg CCK although their CCK-induced suppression of food intake is equivalent to sham vagotomized controls (SHAM). The RT (^{o}C) of the VAGX and SHAM rats 20 min after ip saline or CCK and the shift in RT from preinjection to the 20 min time point (^{o}C diff) are tabulated below.

	Saline ip		CCK 4 µg ip	
	20 min	^{o}C diff	20 min	^{o}C diff
VAGX	37.7±0.0	0.5±0.1	37.5±0.1	0.4±0.1
SHAM	37.6±0.1	0.5±0.1	37.0±0.1	-0.1±0.1*

[means ± SE; * RT significantly (p<.01) less than 20 min SHAM and CCK saline and VAGX CCK day]

In tests with a preferred food present, there was no difference in percent suppression of food intake in response to 4 µg/kg CCK between VAGX (93±4%) and SHAM (96±1%) groups. Our findings indicate that the reduction of body temperature by CCK is mediated by vagal neurons which appear to be distinct from the vagal neurons mediating suppression of food intake.

375.5

CRF-MICROINFUSION INTO THE PARAVENTRICULAR NUCLEUS OF THE HYPOTHALAMUS (PVN) HAS DUAL EFFECTS ON BEHAVIORAL PARAMETERS IN RATS.
H. Mönnikes*, I. Heymann-Mönnikes* and Y. Taché.
CURE/VA Wadsworth Medical Center, Dept. of Medicine and Brain Research Institute, UCLA, Los Angeles, CA 90073.

CRF injected ICV has dual dose-related effects on behavior in rats (increased locomotion at low and freezing-behavior at high doses). CRF injected into the PVN increases locomotion and grooming but whether the PVN is involved in mediating these dose-related behavioral responses to central CRF is unknown. Therefore, in this study we assessed if different CRF doses microinfused into the PVN mimick the ICV-effects of the peptide on behavior. 90 SD-rats were implanted with a 22G guide cannula 3 mm above the PVN or the lateral hypothalamus (LH). 7 days later, in fasted freely moving rats, CRF or vehicle was microinjected (100 nl) via a 30G cannula. Behavioral parameters were monitored 15 min later for 45 min in a familiar environment. Results: (mean±SEM; $^{(1)}p<0.01$, $^{(2)}p<0.001$, $^{(3)}p<0.005$; t-test). CRF microinjected into the PVN increased grooming dose-dependently from 12±4 (vehicle) to 60±15$^{(3)}$ (0.06 nmol), 69±11$^{(2)}$ (0.2 nmol) and 114±2$^{(2)}$ (0.6 nmol). Locomotion increased from 19±4 to 40±9$^{(1)}$ (0.06 nmol) whereas at the higher dose (0.6 nmol) it decreased to 3±2$^{(3)}$ and freezing-behavior occured in 30%. CRF (0.2 nmol) microinfused into the LH had no effect on these parameters. These data show that the PVN is a sensitive site for the dose-related and dual alterations of behavior induced by CRF. This results and previous studies (Gastroenterology 98: A512, 1990) suggest, that the PVN is an important site to integrate both behavioral and visceral effects of CRF.
Supported by NIH, DK-33061 and the Deutsche Forschungsgemeinschaft.

375.6

IN VIVO EVIDENCE FOR A NEUROTENSIN/ADENOSINE INTERACTION IN CNS.
F.B. Jolicoeur and D. Ménard. Departments of Psychiatry and Pharmacology, University of Sherbrooke, Faculty of Medicine, Sherbrooke, Québec, Canada, J1H5N4.

Although much emphasis has been placed on the interaction of neurotensin and dopaminergic transmission, it has become increasingly evident that the peptide interacts with a multiplicity of transmitters. To our knowledge the possibility that "purinergic transmission" is involved in neurotensin's central actions has not been examined previously, and is the focus of the present study. This was prompted by the observation that the neurobehavioral effects of adenosine agonists are strikingly similar to those produced by neurotensin.

In preliminary experiments we determined that the IP administration of 10 mg/kg of the adenosine antagonist, theophylline, did not in itself affect either body temperature or motor activity of rats. We then examined the effects of pre-administration of this dose of the drug 30 min prior to the ICV administration of several doses of neurotensin (3.75-30.0 µg) on hypoactivity and hypothermia induced by the peptide.

Results indicate that theophylline completely blocked or significantly attenuated the hypothermia induced by 3.75 and 7.5 µg neurotensin respectively. The decrease in body temperature induced by larger doses of the peptide were not affected by the adenosine antagonist. For motor activity, theophylline systematically abolished the hypoactivity produced by 3.75, 7.5 and 15 µg neurotensin. On the other hand, the reduction in activity induced by 30 µg of the peptide was not significantly affected by theophylline.

Although the exact nature of theophylline's blockade of neurotensin's central actions remains to be determined, these early results point to a neurotensin/adenosine interaction.

Supported by the Medical Research Council of Canada

375.7

NEUROTENSIN IN CHRONIC NEUROLEPTIC EFFECTS.
A. J. Stoessl & E. Szczutkowski*. Dept. of Clinical Neurological Sciences, Univ. of Western Ontario, London, Ontario, Canada.

Chronic neuroleptic administration produces a syndrome of vacuous chewing mouth movements (VCM's) in rodents, which may serve as a model for tardive dyskinesia, and also results in elevations of striatal neurotensin (NT)-like immunoreactivity and nigral NT binding. We examined the effects of NT manipulations on chronic neuroleptic-induced VCM's in the rat. Male Sprague-Dawley rats received fluphenazine (FLU; 12.5 mg/kg) or its vehicle (VEH; sesame oil) IM every 3 weeks for 18 weeks, and beginning 4 weeks after the last injection of FLU or VEH, were then observed continuously for 60 min. following the intracerebroventricular administration of NT (1ug, 2ug), the NT antagonist D-Trp^{11}-NT (1ug, 2ug, 5ug), or their vehicle (0.9% saline). NT significantly increased the duration of VCM's in both FLU and VEH groups ($p<0.001$), but the effect was more pronounced in animals treated with FLU. Although there was a tendency for D-Trp^{11}-NT to suppress VCM's in both groups, this effect did not reach significance. Locomotion was decreased in animals treated with FLU ($p=0.001$ in NT experiment, 0.003 in D-Trp^{11}-NT experiment), but neither NT nor D-Trp^{11}-NT modified this response. These results are compatible with a contribution of NT to the dyskinetic effects of chronic neuroleptics, but further delineation will require the development of better NT antagonists.

375.8

ASTROCYTIC BENZODIAZEPINE RECEPTORS AND THEIR ENDOGENOUS LIGANDS IN HEPATIC ENCEPHALOPATHY.
J.F. Giguere[1], J. Lavoie[1], M.C. Tonon*[1], L. Desy*[3], H. Vaudry*[2], G. Pelletier[3] and R.F. Butterworth[1], [1]Lab. of Neurochemistry, CRC A-V. Hôpital St-Luc, Montreal, Que., [2]Lab. Endocrinol., Université Rouen, France, [3]Groupe Endocrinol. CHU, Laval, Que., Canada.

There is evidence to suggest that benzodiazepine receptors or their endogenous ligands may play a role in the pathogenesis of hepatic encephalopathy (HE). Although central GABA-related benzodiazepine receptors are unchanged in HE, previous studies have shown that astrocytic "peripheral-type" benzodiazepine receptors (PTBR's) are significantly increased in density in postmortem brain tissue from cirrhotic patients who died in hepatic coma (Lavoie et al., Hepatology (1990). Following portacaval anastomosis (PCA) in the rat, binding sites for the PTBR ligand ^{3}H-PK 1195 were found to be similarly increased 2 to 3 fold in brain without concomitant changes of binding affinities. Diazepam Binding Inhibitor (DBI) is an endogenous ligand for benzodiazepine receptors, found both in astrocytes and neurons. Tryptic digestion of DBI yields Octadecaneuropeptide (ODN). The localization of ODN in brain was evaluated by immunohistochemical techniques using an ^{125}I-antibody of high specificity. Following PCA, ODN-immunoreactive material was found to be increased 3-fold in astrocytes of several brain regions. These findings suggest a role for astrocytic benzodiazepine receptors in the pathogenesis of experimental and human HE. (Funded by MRC Canada)

375.9

VASOACTIVE INTESTINAL POLYPEPTIDE (VIP) IS A SECRETAGOGUE FOR PROTEASE NEXIN I (PNI) RELEASE FROM ASTROCYTES. B. W. *Festoff, J. S. Rao,* and D.E. Brenneman*, Department of Neurology, University of Kansas Medical Center, Kansas City, KS 66103, and Neurobiology (151), DVA Medical Center, Kansas City, MO. 64128. *LDN, NICHD, NIH, Bethesda, MD.

Communication between neurons and glia is a fundamental process underlying nervous system development. Signals arising from either cell type have critical actions on the other. VIP has been shown to increase survival (PNAS 83: 1159-1162, 1986) mediated through glial cells (JCB 104, 1603-1604, 1987). VIP, a known secretagogue, may enhance the release of glial-derived neuron survival factors. We now investigate if VIP may promote secretion of the serpin, GDN/PNI, from astrocyte cultures. Seventy percent confluent new born rat cortical astroglial cultures were incubated for 3 days with ^{35}S-methionine. Following the removal of excess label, VIP, VIP inactive analog, or somatostatin (at 0.1 nM) were incubated for 3 hours. The labeled medium was removed and incubated with non-immune serum or monospecific IgG against 47 kDa PNI. VIP doubled the proteins compared to control or VIP analog cultures, while somatostatin reduced it by fifty percent. Autoradiography showed a specific 3-fold precipitation of PNI by anti-PNI only after VIP treatment. These results indicate that VIP is a potent secretagogue of GDN/PNI which may have a fundamental influence on neuronal development.

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375.10

THE ISOLATED EYESTALK OF THE CRAYFISH MAINTAINS A CIRCADIAN RHYTHM OF NEUROSECRETION. L. Rodríguez-Sosa*, J. Calderón*, J. Hernández and H. Aréchiga. Depto. de Fisiología, Biofísica y Neurociencias and Depto. de Biología Celular, CINVESTAV-IPN. Apdo. Postal 14-740, 07000 México, D.F.

The crustacean eyestalk is a putative circadian neurosecretory pacemaker. However, its capability to maintain a self-sustained circadian rhythm in vitro has not been demonstrated. In this work we report the persistence of a circadian rhythm in the amount of a neuropeptide in the isolated eyestalk of the crayfish Procambarus clarki.

As a marker of neurosecretion we used the Red Pigment Concentrating Hormone (RPCH) which was quantified. a) by immunoenzymatic assay (ELISA), with an antibody raised in our laboratory. b) by HPLC, and c) by bioassay on isolated crayfish epithelia. Isolated eyestalks were kept in organ culture during the experiment, in 20%Leibovitz medium, with 1% fetal calf serum. While in culture, they were subjected either to continuous darkness (D:D), cycles of 12:12 hrs. of light and darkness (L:D), or continuous light (L:L). In isolation the RPCH amount in the eyestalk varied in a circadian manner within a range of $2x10^{-12}$ gr to $8x10^{-9}$ gr, with maximum during night phase. τ was 25.5 $\pm$ 1.5 L:L; and 25 $\pm$ 1 in D:D.

375.11

TEMPERATURE MODULATES THE REGULATORY ACTIONS OF ALPHA BAG CELL PEPTIDE. R. Sanger Redman and R.W. Berry. Dept. Cell, Molecular, and Structural Biology, Northwestern Univ., Chicago, IL 60611.

Egg-laying in Aplysia californica is seasonal, occurring mainly in the summer, and is caused by a peptide egg-laying hormone (ELH) released during a discharge by the bag cells. Alpha bag cell peptide (α-BCP), one of the peptides secreted along with ELH, is suspected to play an autoregulatory role in modulating the characteristics of the discharge. The feedback effects of α-BCP have been reported as autoexcitatory by some and autoinhibitory by others, in experiments performed at 14°C and room temperature respectively. These temperatures approximate winter and summer water temperatures experienced by the animal; thus the effect of α-BCP may be temperature-dependent and may be a factor in determining the seasonality of egg laying. To test this possibility, α-BCP's effect on bag cell membrane potential was determined. At 15°, α-BCP caused a hyperpolarization, whereas at 20°, the peptide caused a small depolarization. Extracellular recordings revealed that at 15° α-BCP shortened the duration of the bag cell discharge and decreased the total number of action potentials generated. At 20°, the peptide lengthened the discharge and increased the number of action potentials. A transient rise in cAMP is associated with the discharge; therefore, we measured α-BCP's effect on bag cell cAMP levels at various temperatures throughout the animal's natural environmental range (12°-22°). The influence of α-BCP is highly temperature-sensitive. The function of the peptide reversed from inhibition (12-15°) to stimulation (17.5-22°) over a 2.5° range. These results imply that α-BCP is temperature-sensitive, functioning in an autoinhibitory manner at 15° and becoming autoexcitatory at 20°. Thus, it may function in coordination with other factors to control egg-laying in response to seasonal temperature variations.

INGESTIVE BEHAVIOR: MONOAMINES

376.1

Histidine, Histamine, and Amino Acid Neurotransmitter Dynamics of Hypothalamic Nuclei in Meal fed Rats. Dodds, S.J., L.P. Mercer* and J.D. Dunn. Dept. of Biochem. Oral Roberts Univ. Sch. of Med., Tulsa, OK 74171.

Neurotransmitter patterns are assessed under regimented meal conditions and compared to previous findings of elevated histidine in protein energy malnutrition.

Quantitive experiments were preformed to evaluate histamine (HA), Gly, Tau, GABA, Glu, Asp, levels of hypothalamic nuclei, in relation to meal fed rats.

In meal regimented rats, his dynamics of the lateral hypothalamus (LH) and ventromedial hypothalamus (VMH) are inversely related. Over the course of the meal, high VMH his and low LH his reflect hunger, and low VMH his and high LH his correlate with satiated states. In contrast no significant dynamics are demonstrated with HA, however VMH HA decreases with increasing hunger in the premeal (ac) period. Median Eminence/Arcuate nuclei (ME) reveal increasing HA with increasing satiety. The paraventricular nucleus (PVN) was found to have elevated HA in ac period. Levels fall significantly during the ingestive phase and return to ac levels 1 hour postmeal. Putative amino acid neurotransmitter dynamics were also found to correlate anatomically with food intake changes.

Data is obtained by microdissection of freeze-dried brain sections, obtained at appropriate time interval. Amino acid analysis is done by HPLC methodogy and HA levels are obtained with a radioimmunoassay.

376.2

INDEPENDENT INGESTION OF WATER AND MILK BY PRE-WEANLING RAT PUPS FOLLOWING SYSTEMIC HISTAMINE. Steven M. Specht, Robyn M. Cashmore* and Kevin B. Dempsey*. Psychology Department, Lebanon Valley College of Pennsylvania, Annville, PA 17003.

An independent ingestion paradigm, in which pre-weanling rat pups are allowed to consume fluid from the floor of a test chamber, was used to assess intake levels in 13 day-old rat pups. Each pup received a subcutaneous injection of either: 1) 0.15M NaCl; 2) 1.0M NaCl; 3) 40 mg/kg histamine (HA) or 4) 80 mg/kg HA and was placed in a test chamber (35°C) for 30 min with either water or milk spread on the floor. Although pups exhibited increased water intake following hypertonic 1.0M NaCl, there was no apparent increase in water intake following either dose of HA. Pups in all treatment groups exhibited similar milk intake levels during the 30 min test. These results suggest that 1) the independent ingestion procedure may be useful to examine *water* intake following homeostatic challenges in pre-weanling rat pups, and 2) there may be a relative insensitivity to the dipsogenic effects of HA for pups at this age tested with this procedure. These results are consistent with the finding that weanling rats are less sensitive than adults to the dipsogenic effects of HA, requiring higher doses of HA than adults to elicit drinking (20 vs. 5 mg/kg, respectively)(Specht, S.M. and Spear, L.P., Physiol. Behav., 45:63, 1989).

376.3

DEVELOPMENTAL PATTERNS OF NUTRIENT INTAKE, SPECIFIC APPETITES AND BODY WEIGHT: RELATION TO HYPOTHALAMIC α_2-NORADRENERGIC RECEPTOR SITES AND CIRCULATING LEVELS OF GLUCOSE AND INSULIN. D.J. Lucas*, M. Jhanwar-Uniyal, K. Leibowitz*, Y.S. Jhanwar* and S.F. Leibowitz. The Rockefeller University, New York, N.Y. 10021

In female (n=23) and male (n=18) rats, raised on pure macronutrient diets from birth, measurements were taken of body weight (BW, days 1-77), post-weaning 24hr nutrient intake (days 21-77), 10-min intake of sucrose (8%) or half & half (10.5%) solution (days 30-50), and blood levels of glucose and insulin or hypothalamic α_2-noradrenergic (3H-p-aminoclonidine) receptor binding sites measured at sacrifice (day 77). The main findings are: (1) BW 3 days after birth, in male and female rats, is positively related to the rats' preference (measured from days 21-77) for carbohydrate (but not protein or fat) and for sucrose. (2) At weaning (day 21), carbohydrate and fat intake (for females) and protein, carbohydrate and fat intake (for males) is positively related to the rats' nutrient intake patterns at maturity (day 77). (3) At puberty (days 40-50), females exhibit a strong preference for carbohydrate and males for protein. In both sexes, 24hr carbohydrate intake is correlated with appetite for sucrose, whereas preference for half & half reflects fat intake. In female rats, a positive relationship exists between BW and appetite for sucrose or half & half at puberty. (4) At maturity, BW is strongly related to appetite for sucrose (days 30-50), fat intake (days 21-77), and blood levels of insulin (day 77). (5) α_2-noradrenergic receptor sites, in the paraventricular nucleus (PVN) of female rats and dorsomedial nucleus (DMN) of male rats, are positively related to measures of protein intake and BW, while these measures are inversely related to α_2 receptors in the ventromedial nucleus (VMN) and perifornical lateral hypothalamus (PLH) of female rats only. Circulating glucose is positively related to α_2 receptors in the PVN, VMN and DMN but inversely related to α_2 receptors in the PLH. These results reveal a close relationship between nutrient intake and appetite, BW, circulating hormones and α_2 receptors in these hypothalamic sites.

376.4

α_2-NORADRENERGIC RECEPTOR AGONISTS AND ANTAGONISTS HAVE OPPOSITE EFFECTS ON CARBOHYDRATE AND PROTEIN INGESTION IN THE RAT. W.K. Cheung*, C.B. Dietz*, J.T. Alexander*, J. Ade*, G. Brennan and S.F.Leibowitz. Rockefeller Univ, N.Y., N.Y.10021

α_2-Noradrenergic receptors in the paraventricular nucleus (PVN) of rats are believed to have opposite effects on carbohydrate and protein ingestion at the onset of the active (dark) cycle. To examine the physiological role of α_2 receptors in controlling natural patterns of nutrient intake at this time, experiments with the selective α_2-receptor antagonists, idazoxan (IDA) or rauwolscine (RAU), and the α_2 agonist clonidine (CLON) were conducted in Sprague-Dawley rats.

Tests with i.p. injection of IDA or RAU (0.25-1.0 mg/kg) and 90-min measurements of nutrient intake (protein, carbohydrate and fat) demonstrate that these antagonists produce dose-dependent effects on carbohydrate and protein intake opposite to those observed with CLON or norepinephrine (NE). Whereas carbohydrate ingestion is stimulated, and protein intake inhibited, by PVN NE or CLON injection, carbohydrate intake is either reduced or unaffected by i.p. IDA and RAU, while protein ingestion is potentiated. The rats' relative preferences for these diets (% concentration of total intake) reveal a reliable decrease in appetite for carbohydrate and a small increase in protein appetite. Fat consumption is unaffected by IDA but is increased by RAU, resulting in a significant potentiation of total Kcal intake. However, tests with RAU injection directly into the PVN (10-25 pmols) show a reliable suppression of carbohydrate intake, consistent with this drug's effects after i.p. injection, but fail to reveal the stimulatory effect on fat ingestion, suggesting that it is mediated through a different neural system. Additional tests with i.p. CLON confirm earlier results demonstrating that α_2-noradrenergic stimulation increases the number of carbohydrate-rich meals at dark onset, while protein meals are strongly inhibited. These findings support the proposal that PVN α_2 receptors are physiologically active in balancing patterns of carbohydrate and protein intake at the onset of the active period.

376.5

CLONIDINE DIFFERENTIALLY AFFECTS MACRONUTRIENT INTAKE IN GENETICALLY OBESE AND LEAN MICE: DOSE-EFFECT RELATIONSHIPS. **P.J. Currie and L.M. Wilson. Psychol.Dept., Univ.Manitoba, Winnipeg, Canada, R3T2N2.**

Clonidine [CLON], the α2-noradrenergic agonist, reduces total energy intake primarily through suppression of carbohydrate [CHO] and fat consumption in both genetically obese [*ob*] and lean mice at doses of .1 and .5 mg/Kg (Currie, P.J., Wilson, L.M., *Neurosci. Abstr. 14*:966, 1988). Lower doses, however, selectively potentiate CHO intake, but only in *ob* mutants (Currie, P.J., Wilson, L.M., *Int.J.Obes.* 13:550, 1989). This study extended the dose-effect curve for clonidine [CLON] in *ob* and lean mice: [1] to identify an optimal dose for intake potentiation in *ob*s; [2] to determine whether CLON also exerts a bidirectional dose-dependent effect on CHO intake in lean mice.

After adaptation to a 6-h feeding regimen, *ob* and lean mice [*n*=14/genotype] were injected IP with .15 M NaCl for 2 days 30 min before simultaneous access to CHO, fat, and protein. On the 3rd day, mice were injected with either .025 or .05 mg/Kg CLON or an equivalent volume of .15M NaCl 30 min before meal access. The lower dose augmented *ob*s' total caloric intake and calories obtained from fat [expressed as % of vehicle control intakes] compared to leans', especially within the 1st h of food access [*Genotype X Dose X Time:* $p<.05$,$p<.01$, respectively]. CLON potentiated CHO intake in *ob*s at both doses at 1 h; the effect declined by 6 h. The lower dose did not affect lean mice's CHO intake, but the higher dose suppressed 1-h intakes, as had doses of .1 and .5 mg/Kg in previous research [*Genotype X Dose X Time:* $p<.025$]. With previous results, these comparisons suggest enhanced pharmacological sensitivity of an α-noradrenergic mechanism in genetic obesity. [*Supported by NSERC #7907 to LMW and MHRC Graduate Studentship to PJC]*

376.6

MECHANISM OF TYROSINE(TYR) POTENTIATION OF MIXED-ACTING SYMPATHOMIMETIC ANOREXIANTS (MASA). K.M.Hull* and T.J.Maher. Dept. Pharmacol. Mass.Coll. Pharm., Boston,MA 02115.

We previously reported a TYR specific potentiation of the MASA (±)-phenylpropanolamine (PPA), (+)-amphetamine and(-)-ephedrine (EPH). Thus we examined the dependence on functioning TYR hydroxylase (TH), brain TYR and the release of catecholamines (CA) in this potentiation. To determine if the potentiation by TYR required TH-mediated conversion for CA synthesis, we pre-treated with α-methyl-p-tyrosine (αMPT) and then administered EPH (20mg/Kg) with or without TYR (200mg/Kg). αMPT blocked both the anorectic activity of EPH and its potentiation by TYR in hyperphagic rats. To determine the requirement for central TYR we coadministered equimolar valine (VAL), which competes with TYR for uptake into brain, to food-deprived rats, TYR, or VAL+TYR with either saline (SAL) or PPA (20 mg/Kg). Neither VAL, TYR or VAL+TYR altered food intake when administered to SAL pretreated animals. While VAL alone failed to alter PPA-induced anorexia, VAL+TYR attenuated the normally observed pot-entiation by TYR. We also administered two add-itional MASA: (+)-norpseudoephedrine, (+)-pseudoephedrine, and two direct-acting agonists: salbutamol and methoxyphenamine. Only the MASA were potentiated by TYR. These results suggest that TYR's potentiation results from a central action involving increased CA synthesis via TH.

376.7

THE EFFECTS OF 1-(2,5)-DIMETHOXY-4-IODOPHENYL-2-AMINOPROPANE (DOI) ON THE MEAL PATTERNS OF RATS R. Bauman* and R. Pastel. Dept. of Medical Neurosciences, Walter Reed Army Institute of Research, Washington, D.C. 20307.

The meal patterns of non-deprived rats were used to evaluate the effects of DOI, a $5HT_2$ agonist, on food intake. A 12 hour light/dark cycle was maintained in each rat's cage and at any time a rat could eat by pressing a lever once for each 45 mg food pellet. A meal was defined as a bout of eating that was not interrupted by a pause longer than 10 mins. Thirty minutes before the onset of darkness, either DOI or saline was delivered IP. A maximum dose of 1 mg/kg was used because in a prior study, it reduced the total intake of deprived rats by 50%.

Meal frequency and total food intake were greater during the dark than during the light. A 1 mg/kg dose but not a 0.3 mg/kg dose of DOI significantly reduced the size of a meal (pellets/meal) and total intake during the dark without affecting meal frequency or rate of eating pellets. Meal size was reduced for the first several meals of the dark period. These data suggest that serotonin is an important determinant of meal patterning and total intake in the rat.

376.8

OPPOSITE EFFECTS ON INGESTIVE BEHAVIOR FOLLOWING CHRONIC MUSCIMOL ADMINISTRATION INTO THE MEDIAN RAPHE OR VENTRAL TEGMENTAL AREA, T.R. Stratford and D. Wirtshafter, Dept. Psychology and Committee on Neuroscience, University of Illinois at Chicago, Chicago, IL., 60680.

Previously, our lab has demonstrated large increases in food and water intake in rats during the 2 hours following acute microinjections of the GABA-A agonist muscimol into the median raphe (MR). Smaller increases were observed following similar injections into the VTA. In order to assess the effects of chronic administration of muscimol into these brain areas, Alzet osmotic minipumps were attached to injector cannulae terminating in either the MR or the VTA and were implanted using ether anesthesia. The muscimol was administered in a dose of 25ng/0.5ul at an infusion rate of 0.5 ul/hour.

Animals receiving the muscimol infusion in the MR exhibited extremely large increases in water intake and, to a lesser extent, food intake, along with a severe disruption of the normal day/night ingestion cycle. The hyperdipsia and hyperphagia appeared within a few hours of the minipump implantation and seemed to continue for the life of the pump (7-9 days). Peak 24 hour water intakes were in the 280-350 ml range with food intakes in excess of 40 g for the same period. In contrast, chronic infusions of muscimol into the VTA lead to a complete aphagia and adipsia which was completely reversed within a few hours of terminating the infusion. (Supp. by NS21350)

376.9

THE EFFECT OF INTRAPERITONEALLY ADMINISTERED ICS 205-930 AND QUATERNIZED ICS 205-930 ON FOOD INTAKE OF RATS FED AN AMINO ACID IMBALANCED DIET.

B.J. Hrupka*, D.W. Gietzen, Q.R. Rogers.
Dept VM:Physiol. Sci. and Food Intake Lab, Univ. Calif., Davis, CA, 95616.

We have previously reported that the anorectic response of rats to an amino acid imbalanced diet (IMB) is blocked by intraperitoneal (ip) injections, but not intracerebroventricular injections of the serotonin$_3$ ($5HT_3$) antagonist ICS 205-930 (ICS). ICS has been shown to cross the blood brain barrier (BBB), while quaternized ICS (Q-ICS) should not. Since evidence indicates that the feeding response to IMB is mediated centrally, we used ip administration of ICS and Q-ICS to determine whether the $5HT_3$ receptors that are involved in the rat's response to IMB are located centrally or peripherally. Male Sprague-Dawley rats (n=7 rats/gp) were prefed a low protein basal diet for 10 days. Rats received ICS, Q-ICS, (7.8 or 28.1 μmoles/kg body weight) or saline within 30 min of onset of the dark cycle, and were then fed IMB. Food intake was recorded after 3, 6, 12, and 24 hr. From 0 to 6 hr, rats that received ICS and Q-ICS injections ate similar amounts of IMB (P>.10). Rats injected with 28.1 μmoles/kg ICS and Q-ICS ate significantly more IMB (P<.01) from 6 to 24 hr than rats injected with 7.8 μmoles/kg of these drugs, demonstrating a dose-dependent response to these $5HT_3$ antagonists. There were no significant differences (P>.10) between ICS and Q-ICS in IMB intake. If Q-ICS does not cross the BBB, these results suggest that a major portion of the effect of ICS in blocking the anorectic response to IMB is mediated peripherally. Supported by NIH DK 07355, USDA CRCR 1-2418, CNRU DK35747 and a gift from Sandoz Research Institute.

376.10

SUPPRESSION OF FOOD INTAKE BY MK212 AND OTHER ARYLPIPERAZINES IN FOOD-DEPRIVED RATS. S. M. Snyder*, L. R. Reid*, J. L. Buelke-Sam, and D. T. Wong.
Toxicology Division and Central Nervous System Research, Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN 46285.

The arylpiperazine class of serotonin (5-hydroxytryptamine, 5HT) agonists is known to suppress food consumption in rats[1]. In the present study, food-deprived male Sprague-Dawley rats were treated with 0.3, 1.0, 3.0, or 10.0 mg/kg i.p. doses of either quipazine, m-chlorophenylpiperazine, or MK212. A dose-related suppression of food intake was observed in all groups at 1 and 3 hours of food access. The anorectic effect of MK212 at 10 mg/kg was reversed by pre-treatment with 0.3-5.0 mg/kg i.p. of metergoline (a non-selective antagonist of 5HT-1 and 5HT-2 receptors), 3-10 mg/kg i.p. of mianserin or 3-10 mg/kg i.p. of LY53857 (antagonists of 5HT-1C and 5HT-2 receptors). The anorectic effect of MK212 was not reversed by pre-treatment with 1-3 mg/kg i.p. of ketanserin (an antagonist of the 5HT-2 receptor). At the highest doses used, the antagonists, except LY53857, alone suppressed food intake. The present findings, however, are consistent with the involvement of the 5HT-1C receptor mediating the anorectic effect of MK212. [1]David T. Wong and Ray W. Fuller, Serotonergic Mechanisms in Feeding, International Journal of Obesity (1987) 11, Suppl. 3, 125-133.

376.11

INHIBITORY EFFECTS OF 5-HYDROXYTRYPTOPHAN ON FEEDING IN HUNGRY RATS. C.T. Tsai. Department of Biology, National Changhua University of Education, Changhua, Taiwan, ROC

The effects of the serotonin precursor, 5-hydroxytryptophan (5-HTP) on food intake in free-feeding and food deprived rats were examined. In free-feeding rats, 5-HTP (100 mg/kg, i.p.) reduced food intake by 86.3%. The anoretic effect of 5-HTP was antagonized by cyproheptadine (CYP, 4 mg/kg, i.p.), a serotonin blockade. However, following food deprivation, the food intake was increased 24.3% in control rats, whereas, the food intake was decreased 42.8% in 5-HTP treated rats. That is, the feeding of hungry rats was still inhibited by 5-HTP. Nevertheless, this inhibition of 5-HTP on food intake in hungry rats was almost recovered by CYP. The evidences indicated the anoretic action of 5-HTP in free-feeding and in hungry rats were mediated by serotonergic mechanism. As to analysis of meal patterns, the 5-HTP treated hungry rats ate only 0.16 gms in the initial 2 hr session and took 3.74 gms in the later 6 hr period, while the saline controls ate 10.3 gms in the initial 2 hr session and 2.1 gms in the later 6 hr period. This opposite result apparently showed that the effect of 5-HTP on feeding was inhibition of the initial phase of hungry period. These evidences are in favor of a conclusion that there is an existence of a serotonergic mechanism in brain to be inhibitory for feeding via inhibition of hunger.

376.12

CAN 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN (TCDD)-INDUCED APPETITE SUPPRESSION BE OVERCOME BY ALTERATIONS OF CENTRAL SEROTONIN LEVELS? B.U. Stahl*, R.H. Alper, E.J. Walaszek and K. Rozman*. Dept. Pharm. Tox. and Therap. Univ. Kansas Med. Center, Kansas City, KS 66103 (USA), and Section of Environ. Tox., Ges. f. Strahlen und Umweltforschung mbH München, D-8042 Neuherberg (FRG).

There is evidence that the appetite suppression which is the apparent cause of TCDD-induced death, may be a result of reduced activity of gluconeogenic enzymes followed by an elevation of serum and hypothalamic tryptophan levels and finally resulting in a serotonin (5-HT) mediated reduction of feed intake. Consequently, depletion of central 5-HT should alter the TCDD-induced starvation syndrome. Central 5-HT depletion was accomplished by using i.c.v. infusions of the neurotoxin 5,7-dihydroxytryptamine (5,7-DHT, 150 μg of free base per 20 μl 0.9%NaCl containing 0.1% ascorbic acid). Rats (n=5) were pretreated with desipramine (25 mg/kg i.p.) 30 min. before i.c.v. infusion. Two weeks after infusion these rats and a group of non-lesioned rats (n=3) were treated with a lethal dose of TCDD (125 μg/kg i.p.). Three naive control rats were injected with vehicle alone (corn oil, 4 ml/kg i.p.). All rats were monitored daily for feed intake and body weight development. On day 13 after treatment all rats were sacrificed and 5-HT and its metabolite 5-hydroxyindoleacetic acid (5-HIAA), dopamine (DA) and its metabolite dihydroxyphenylacetic acid (DOPAC) were quantitated in the hypothalamus using HPLC with electrochemical detection. In 2 out of 5 of the 5,7-DHT lesioned animals hypothalamic 5-HT was depleted more than 90% and 5-HIAA more than 85% compared to control animals. In the remaining 3 animals depletions were between 30% and 61% for 5-HT and 36% and 57% for 5-HIAA. No alterations were found in the hypothalamic levels of DA and DOPAC. No differences were observed regarding body weight development and feed intake in TCDD treated rats with or without central depletion of 5-HT. These results suggest that although TCDD increases central 5-HT levels as a result of increased plasma tryptophan, this may not be the sole cause for reduced feed intake and lethality. In further studies it needs to be determined whether elevations in peripheral tryptophan and/or 5-HT can cause TCDD induced starvation.

376.13

THE EFFECT OF A SUBLETHAL DOSE OF TCDD ON THE TURNOVER OF BIOGENIC AMINES IN SUBREGIONS OF RAT BRAIN. M. Unkila[1], L. Tuomisto[2], R. Pohjanvirta[1], E. MacDonald[2] and J. Tuomisto[1], National Public Health Instit., Dept. of Environ. Hyg. & Toxicol., P.O.B. 95, SF-70701 Kuopio, Finland, [2]Dept. of Pharmacol. & Toxicol., Univ. of Kuopio, Kuopio Finland.

Male Han/Wistar rats given a sublethal dose of TCDD (1000 ug/kg ip in corn oil) showed disturbed metabolic control of food intake and lowered body weight for months after TCDD administration (Pohjanvirta et al., in this abstract book). In the present study we examined whether TCDD is interfering with brain aminergic systems, well known to participate in the control for energy homeostasis. 3 1/2 Months after TCDD exposure the rats were given alpha-methyl-p-tyrosine (AMPT; 250 mg/kg ip) and 4 hr after AMPT or vehicle injection their catecholamine (CA) or indoleamine (IND, only AMPT-controls) concentrations were measured by HPLC-EC. TCDD had only minor effects on brain monoamines. The turnover of CA:s was accelerated about 5-10 % in almost all brain areas in the TCDD group. The steady-state concentration of CA:s and IND:s were unaffected as well as of tryptophan which is usually elevated in fasted rats. These results support our previous findings suggesting only a minor or secondary role for the brain monoamines in TCDD toxicity.

376.14

2,3,7,8-TETRACHLORODIBENZO-p-DIOXIN (TCDD) ENHANCES RESPONSIVENESS TO SATIETY SIGNALS AND CAUSES PERSISTENT ALTERATIONS IN RESPONSES TO FEEDING REGULATORY CHALLENGES IN HAN/WISTAR RATS. R. Pohjanvirta* and J. Tuomisto. Natl. Public Health Inst., Dept. of Environ. Hyg. & Toxicol., P.O.B. 95, SF-70701, Kuopio, Finland.

A single sublethal dose of TCDD (1000 or 3000 μg/kg ip) led to persistent hypophagia along with permanent retardation of growth in Han/Wistar rats. In one-bottle tests, these rats showed diminished sucrose drinking accompanied by a progressive tendency toward increased saccharin consumption over control levels. In contrast to control rats, they did not display hyperphagia in response to cellular glucopenia by 2-deoxyglucose and reduced their feed intake after inhibition of fatty acid oxidation by mercaptoacetate. Their feeding behavioral responses to insulin and naloxone were attenuated. Parenterally administered glucose and (even more so) fructose significantly suppressed starvation-induced eating in TCDD-treated rats while being without effect in controls. These findings argue in favor of a central site of action for TCDD. They also suggest enhanced responsiveness to postingestive satiety signals coupled with insensitivity to metabolic cues to be crucial factors underlying the permanently subnormal body weight maintained by TCDD-treated rats.

376.15

INDEPENDENT INGESTION AND INTRAORAL INFUSION OF 10% SUCROSE PRODUCE DIFFERENT PATTERNS OF CENTRAL DOPAMINE METABOLISM IN 14-DAY-OLD RATS. L. Broder*, G.P. Smith, A. Tyrka*, and J. Gibbs. Bourne Lab, NY Hosp.-Cornell Med. Ctr., White Plains, NY 10605

The D-2 antagonist, raclopride, and the D-1 antagonist, SCH 23390, inhibit intake of 10% sucrose in 14-day-old rat pups when a pup ingests 10% sucrose by licking it from the floor of a tissue-lined beaker (Independent Ingestion, II), but not when a pup ingests 10% sucrose infused into its mouth through an anterior, sublingual, oral catheter (OC; Smith et al, 1989; Tyrka et al, this meeting). The differential potency of the antagonists may be due to a differential effect of the ingestion of 10% sucrose on central dopaminergic (DA) activity in the two tests.

Method: To test this hypothesis, DA, DOPAC and HVA were measured by HPLC-EC in 14-day-old, male and female pups at the end of a 20-min II or OC test.

Results: Ingestion of 10% sucrose by II, but not by OC increased DOPAC/DA in hypothalamus and olfactory tubercle ($p<.05$). Both II and OC increased caudate DOPAC/DA ($p<.05$). In contrast, ingestion of 10% sucrose by OC increased HVA/DA in amygdala and hypothalamus ($p<.05$), but II of 10% sucrose had no effect on HVA/DA in any region.

Conclusions: (1) Regional pattern of DA metabolism depends on the mode of ingestion (II or OC) of 10% sucrose; (2) increased DOPAC/DA in hypothalamus and olfactory tubercle by II, but not by OC, correlates with the inhibitory effect of raclopride and SCH 23390 on II, but not OC; (3) the significant increase in hypothalamic DOPAC/DA produced by II of 10% sucrose in 14-day-old pups extends our observation of this in adult rats (Smith et al, 1987) and suggests that this effect is innate rather than acquired.

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376.16

SCH 23390 INHIBITS INTAKE OF SUCROSE MORE DURING INDEPENDENT INGESTION THAN DURING INTRAORAL INFUSION IN RATS AS EARLY AS POSTNATAL DAY 7. A. Tyrka*, G.P. Smith, and J. Gibbs. Bourne Lab, NY Hospital-Cornell Medical Center, White Plains, NY 10605.

SCH 23390, a D-1 dopaminergic antagonist, decreases the sham feeding of sucrose in adult rats (ID_{50}=60 $\mu g \cdot kg^{-1}$, ip, Schneider et al, 1988). To determine the ontogeny of this effect, intake of 10% sucrose was measured at postnatal days 7, 14, and 21. Pups were removed from the litter for 4h and then placed in a warm, humid chamber for one of two 20-min tests: (1) continuous infusion of 10% sucrose through an anterior, sublingual catheter (Hall, 1979) or (2) independent ingestion of 10% sucrose from the bottom of a test beaker. A dose of SCH 23390 (30-267 $\mu g/kg$) or saline alone was injected ip 15 min prior to an intake test. Each pup was tested only once. SCH 23390 was significantly more potent for decreasing intake in the independent ingestion test than in the intraoral infusion test at each of the three ages (Table).

POTENCY OF SCH 23390 (ID_{50}, $\mu g \cdot kg^{-1}$, ip)

DAY	INTRAORAL CATHETER	INDEPENDENT INGESTION
7	>240	60
14	>229	14
21	>267	67

Although the reason(s) for the differential potency in the 2 tests is not clear, the results suggest that D-1 receptor activity is necessary for the positive reinforcing effects of sucrose on independent eating as early as postnatal day 7. In contrast, D-2 receptor activity is not necessary for independent ingestion until day 14 (Smith et al, 1989).

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376.17

THE INTERPRETATION OF SHAM FEEDING DATA: CURVE SHIFT STUDIES. H.P. Weingarten, A. Duong*, S. Gowans* & D. Elston*. Department of Psychology, McMaster University, Hamilton, Ontario, Canada, L8S 4K1.

Changes of sham feeding are often assumed to reflect alterations of palatability. However, sham feeding is influenced by many performance variables independent of palatability. We adapted the curve shift paradigm, used in the brain stimulation reward field to distinguish reward from performance effects, to sham feeding to discriminate manipulations which alter palatability from those which influence sham feeding performance independent of any influence on taste.

Adult rats sham fed sucrose solutions ranging from .03M to 1.50M. Concentration-intake functions were generated which plotted sham intake (in mls/30 min) versus log of sucrose concentration. Curve shifts were indexed by calculating the log sucrose concentration which produced 50% of asymptotic sham intake, ie the half-max sucrose. Decreasing sucrose palatability by quinine adulteration reduced asymptotic intake by 27% and shifted the concentration-intake function to the right. Half-max values were .89 for unadulterated, and 1.24 for adulterated, sucrose. Dopamine antagonism (.5 mg/kg pimozide) mimicked effects of degrading palatability: reduced asymptotic sham intake with a rightward shift of the concentration-intake function. Pimozide increased half-max sucrose concentration from .7 to 1.1. In contrast, rats sham feeding after 1, 6, 12, or 18 hrs food deprivation showed a two-fold change in asymptotic sham intake but no shift in the concentration-intake functions; half-max sucrose concentrations were identical at all deprivation levels. These data suggest that: i) the effects of a treatment on sham feeding must be assessed by examining the entire concentration-intake function, and; ii) congenial with the conclusion from analogous studies in the brain stimulation reward literature, although many variables alter sham feeding performance, only those treatments which curve-shift operate by altering the motivating capacity of taste.

376.18

EFFECTS OF PIMOZIDE AND AMPHETAMINE ON THE MICROSTRUCTURAL COMPONENTS OF FEEDING. *A.K. Houlihan*, M.L. Volkov*, and S.M. Feldman.* Department of Psychology and Center for Neural Science, New York University, NY, NY, 10003.

Using a discrete trials paradigm, we investigated the effects of systemic pimozide and amphetamine on the microstructural components of the first meal following 24 hour food deprivation in rats. Deprived subjects were adapted to trial-structured meals of wet mash (Purina rat chow and water, 3:2, w/w) and i.p. injections of physiological saline over 11 days. Each animal served in all conditions. Experimental sessions consisted of a maximum of 30 feeding trials, each of 30 sec. duration, separated by a 90 sec. intertrial interval. Food intake was measured following each trial, and bites and pauses were measured on-line within each trial. Sessions were videotaped for subsequent verification of latency; duration; and bite frequency and distribution.

Consistent with previous work in our laboratory, all groups showed a decline in intake rate over the course of a session that was a function of decreasing bite size; bite frequency remained constant over trials. In addition, pimozide (0.5 and 1 mg/kg i.p.) and amphetamine (0.5 and 1 mg/kg i.p.) each decreased cumulative intake in a dose-dependent manner, by both reducing amount eaten per trial and decreasing duration of eating per *session*, compared to vehicle controls. Pimozide also decreased number of trials on which eating occurred, leaving duration of eating per *trial* unaffected. Therefore, pimozide reduced amount eaten per trial by slowing local rate of intake. By contrast, amphetamine reduced intake per trial by decreasing duration of eating within 30 sec. trials, either by increasing latency or pausing, and not by altering local intake rate. The basis for pimozide's reduction of local intake rate is apparently a decrease in bite size, because there was no alteration of local bite rate.

376.19

THE DOPAMINERGIC REGULATION OF FEEDING BEHAVIORS: AN EXAMINATION OF THE INVOLVEMENT OF DISCRETE STRIATAL SUBREGIONS IN THE RAT BRAIN. A.E. KELLEY and V.P. BAKSHI*, Dept. of Psychology, Harvard Univ., Cambridge, MA 02138.

Dopamine (DA) is thought to be critically involved in motor and motivational aspects of behavior. In particular there is considerable evidence that DA may modulate certain components of feeding behavior. Both neuroleptics and amphetamine disrupt feeding, but little is known about the specific striatal subregions involved in these effects. Food-deprived rats were injected with either haloperidol (0.025, 0.25, 2.5 µg) or amphetamine (1, 10, 20 µg) in one of three striatal sites: nucleus accumbens (N.Acc.), ventrolateral striatum (VLS), and dorsolateral striatum (DLS). It was found that food intake, feeding rate, food spillage and feeding durations were differentially affected following drug administration into N.Acc. and VLS. Injections into DLS produced no significant changes in ingestive behaviors. Results were found to be compatible with previous work that indicates a functional heterogeneity of the striatum. Moreover, it is hypothesized from these results that VLS is specifically involved in the regulation of oral motor mechanisms and that N.Acc. may regulate attentional or switching mechanisms that are required for the maintenance of feeding behavior.

377.1

EFFECT OF LITHIUM CHLORIDE AND DESIPRAMINE ON HIV REPLICATION, D.L.Evans, M.S. Smith*, J. Petitto*, R.N. Golden*Depts of Psychiatry & Medicine, and Lineberger Cancer Research Center, Univ of North Carolina, Chapel Hill, N.C.

Lithium and tricyclic antidepressants are used for the treatment of affective disorders in patients with HIV-related illness. Because lithium has immunoactive properties, we studied the effects of lithium chloride (LiCl) and the antidepressant, desipramine (DMI), on HIV replication in order to determine if these agents demonstrate an anti-HIV effect or conversely, an enhancement of HIV replication.

We studied the effect of LiCl and DMI on HIV replication in T-lympochytes in culture, and the effect of LiCl on reverse transcriptase activity in vitro. H9 and C3 cells were infected with the LAV-1 strain of HIV-1 and the production of HIV from drug treated cells was estimated by reverse transcriptase activity.

We found no effect of LiCl (20uM - 20mM) on the activity of HIV to replicate in T cells, as well as no effect of DMI (10 ng per mL - 1 mg per mL) on virus replication. In addition, we found no effect of LiCl (.01 mM-20mM) on the reverse transcriptase enzyme itself.

These preliminary data suggest that neither lithium, nor the antidepressant, DMI, diminish HIV replication. Thus, these findings do not support an anti-HIV role for lithium or DMI. However, and importantly for the clinical management of individuals with HIV infection, these pilot data show no enhancement of HIV replication and therefore do not call into question the use of these agents for the treatment of affective illness in these individuals.

377.2

[3H]OPIPRAMOL LABELS A NOVEL BINDING SITE AND THE SIGMA RECEPTOR IN RAT BRAIN MEMBRANES. D.J. Hirsch, C. D. Ferris, B. P. Brooks, A. M. Snowman, and S. H. Snyder, Dept. of Neuroscience, The Johns Hopkins Univ. Sch. of Medicine, Baltimore, MD 21205.

Opipramol is a tricyclic antidepressant that has a structure which is very similar to the major clinically effective antidepressants. Though its structure is similar to other antidepressants, and though opipramol itself has been used extensively as an effective antidepressant medication, it is virtually inactive when assayed for inhibition of catecholamine uptake (a mechanism though which many antidepressants are thought to exert their therapeutic effects). We have used tritium labeled opipramol to study its high affinity binding to brain membranes. [3H]opipramol labels two sites in brain membranes for which it has equal high affinity (Kd=4-6nM, Total Bmax=3 pmol/mg protein). Haloperidol, which has very high affinity for sigma receptors, differentiates these two site clearly. Haloperidol has a Ki of 1nM for the sigma receptor component of [3H]opipramol binding and a Ki of 350nM for the novel opipramol binding site. Therefore, we have blocked the sigma component with 50nM haloperidol to study this novel site in isolation. Pharmacological characterization has confirmed that [3H]opipramol labels the sigma receptor and that the second binding site is not any known neurotransmitter receptor. Also, several drugs which are relatively inactive at inhibiting catecholamine uptake and yet active as antidepressants are potent inhibitors of opipramol binding to this novel site. We suggest that this site may mediate some of the antidepressant effects of these drugs

377.3

EFFECTS OF CHRONIC IMIPRAMINE ON TYROSINE HYDROXYLASE ACTIVITY IN NORADRENERGIC AND DOPAMINERGIC REGIONS OF RAT BRAIN. D.L. Rosin, A.M. Knorr, E.J. Nestler, R.H. Roth, and R.S. Duman. Depts. of Pharm. & Psych., Yale Univ. Sch. Med., New Haven, CT 06510.

We previously reported that chronic antidepressants produce a decrease in tyrosine hydroxylase (TH) mRNA and in TH immunoreactivity in locus coeruleus (LC) but not in substantia nigra (SN) (McMahon et al., Soc. Neurosci. Abst. 15:986, 1989). To further investigate this finding we measured the enzymatic activity of TH (assayed *in vitro*) in several regions of rat brain after chronic imipramine treatment. In the dopamine cell body regions, TH activity was decreased by 20% in SN (A9) and by 45% in the ventral tegmental area (A10). In an analogous fashion, TH activity was decreased by 20-40% in projection regions of dopamine A10 neurons, i.e. nucleus accumbens and medial prefrontal cortex, and by 30-60% in subregions of the striatum, an area which receives projections from both A9 and A10 cell body regions. Despite previously observed decreases in TH immunoreactivity and mRNA in LC, no significant decrease in TH activity was found in LC, the noradrenergic cell body region, or in the hippocampus, which receives projections from the LC. These results demonstrate that TH activity is diminished in dopamine neurons by chronic imipramine and support a growing body of evidence that antidepressants also alter dopaminergic function. It remains to be seen whether the effects on TH in dopamine regions are relatively more selective for dopamine A10 vs. A9 neurons and whether other noradrenergic regions are affected. (Supported by USPHS MH45481 and MH14092).

377.4

MIANSERIN ATTENUATES LOCUS COERULEUS (LC) ACTIVATION ELICITED BY PHASIC SENSORY STIMULI, HEMODYNAMIC STRESS, AND I.C.V. CORTICOTROPIN-RELEASING FACTOR (CRF): POSSIBLE MODE OF ANTIDEPRESSANT ACTION. A.L. Curtis and R.J. Valentino, Department of Mental Health Science, Division of Behavioral Neurobiology, Hahnemann University, Philadelphia, PA 19102.

CRF hypersecretion has been postulated to occur in depression. Because CRF alters LC discharge characteristics, we hypothesized that antidepressants interfere with CRF effects on LC discharge. Previous results supporting this showed that chronic administration of desmethylimipramine (DMI) attenuated LC activation elicited by nitroprusside infusion, a hemodynamic stress which is thought to require endogenous CRF release for LC activation. To further test this hypothesis, the acute and chronic effects of the atypical antidepressant, mianserin, on LC discharge characteristics were quantified in the present study. Acute i.v. administration of mianserin (0.0001 - 1.0 mg/kg) to halothane anesthetized rats increased LC spontaneous discharge and decreased LC discharge evoked by repeated sciatic nerve stimulation. Mianserin (0.1 mg/kg, i.v.) completely blocked LC activation elicited by hemodynamic stress produced by i.v. infusion of nitroprusside (10 ug/30 ul/min; 15 min). Mianserin also inhibited LC activation produced by i.c.v. administered CRF (3.0 ug in 3.0 ul). Chronic mianserin administration (10 mg/kg, i.p./day for 21 days) resulted in tolerance to its effects on LC spontaneous discharge, LC sensory evoked discharge, and LC activation by i.c.v. CRF. In contrast, LC activation by hemodynamic stress was still greatly attenuated in chronically treated rats. The results indicate that acute mianserin administration attenuates LC activation by a variety of stimuli and that tolerance occurs to some of these effects with chronic administration. Persistent attenuation of LC activation by hemodynamic stress after chronic mianserin administration suggests that interference with putative CRF neurotransmission in the LC may be an important mode of action of mianserin and other antidepressants. This study was supported by PHS Grants MH 42796 and MH 40008 and a NARSAD award to ALC.

377.5

PROPHYLACTIC AND THERAPEUTIC EFFICACY OF ADINAZOLAM IN A RAT MODEL OF DEPRESSION. W. W. Woodmansee, L. H. Silbert*, S. F. Maier and P. H. Desan. Dept. of Psychology, Univ. of Colorado, Boulder, CO, 80309, and Dept. of Neurology, Stanford University, Stanford, CA, 94304.

We have reported that exposure of rats to three sessions of repeated inescapable shock induces a long-lasting period of decreased daily running wheel activity, which is antagonized by chronic administration of desipramine following the stress sessions (Desan, Silbert and Maier, Pharmacol. Physiol. Behav. 30(1988)21-29). In the present study we administered adinazolam 30' prior to each stress session (5 and 10 mg/kg i.p.) or in the days after the stress sessions (5 and 20 mg/100 ml in drinking water). Adinazolam both attenuated the stress-induced activity decrease when given prior to stress, and reversed it when given after stress. As in the case of desipramine, the effect of adinazolam required 7 days for maximum effect, produced activity in excess of normal levels and showed a strongly curvilinear dose response curve. Adinazolam in either pattern of administration had minimal effects on the activity of unstressed animals. In general, anxiolytic agents acutely administered prior to stress reduce the effects of inescapable stress such as learned helplessness, but cannot not reverse them. By contrast, antidepressant agents acutely administered prior to stress fail to prevent such effects, but can reverse them in chronic administration. In the present study adinazolam appeared to have both effects. This result is consistent with clinical reports of both anxiolytic and antidepressant efficacy for this and other triazolobenzodiazepines.

377.6

ACUTE ADMINISTRATION OF THE ANTIDEPRESSANT TRAZODONE INCREASES NORADRENERGIC LOCUS COERULEUS NEURONAL FIRING IN ANESTHETIZED RATS. C. P. VanderMaelen and J. P. Braselton. Bristol-Myers Squibb Co., CNS Biology, Dept. 404, 5 Research Parkway, Wallingford, CT 06492.

Trazodone is an antidepressant drug with moderate 5-HT reuptake blockade properties (IC_{50}=115 nM). It also binds to 5-HT_2 (IC_{50}=11 nM), 5-HT_{1A} (IC_{50}=288 nM), and α_1-adrenergic (IC_{50}=23 nM) receptors (A.S. Eison et al., Psychopharmacol. Bull., submitted). The effects of i.v. administered trazodone were assessed on the spontaneous firing of putative noradrenergic neurons in the locus coeruleus (LC) of male Sprague-Dawley rats anesthetized with chloral hydrate. Standard extracellular single-unit recording techniques using glass microelectrodes were employed. Although considerable variability in responses was observed, overall, trazodone increased the firing rate of noradrenergic LC neurons in a dose-dependent manner when assessed both between cells and within cells. The dose estimated to produce an increase in firing rate of 25% (ED_{25}) was calculated to be 0.128 mg/kg, i.v., and the ED_{50}=0.659 mg/kg, i.v. These results agree generally with Scuvee-Moreau and Dresse (1982, Arch. Int. Pharmacodyn., 260, 299-301), who also observed potent inhibition of serotonergic dorsal raphe neurons with trazodone. The combination of potent inhibition of serotonergic neurons and mild excitation of noradrenergic neurons defines a unique electrophysiological profile for trazodone when compared to other antidepressants.

377.7

RAPID TRYPTOPHAN DEPLETION REVERSES ANTIDEPRESSANT RESPONSE AND ALTERS MOOD IN DEPRESSION P.L. Delgado, D.S. Charney, L.H. Price, G.K. Aghajanian, G.R. Heninger, West Haven VAMC and Dept. of Psychiatry, Yale University School of Medicine, New Haven, CT 06508

Brain serotonin (5-HT) content is dependent on plasma levels of the essential amino acid, tryptophan (TRP). We have previously reported on the effects of rapid dietary TRP depletion in psychiatric patients. This study extends those reports and further characterizes the effects of rapid TRP depletion on mood in depressed patients. METHOD: 87 depressed (DSM-III-R) patients (47 drug free, symptomatic, and 40 in clinical remission on antidepressant) have received tryptophan depletion testing with two 2-day tests each involving a 24-hr., 160 mg/day, low-TRP diet followed the next morning by a 16-amino acid drink, in a double-blind, placebo-controlled (TRP depletion (TD) and control testing), crossover fashion. On one test the diet and drink were supplemented with L-TRP (control) and on the other test neither the diet nor drink were supplemented (TD). Behavioral ratings (Hamilton Depression Scale (HDRS)) and plasma (for TRP levels) were obtained prior to, during and after testing. RESULTS: Total and free TRP decreased 80 to 90% 5 hrs. after the TRP-free drink (TFD). 35% of 47 symptomatic, drug-free depressed patients were unchanged the day of the TFD, but became clinically less depressed (40% mean decrease in HDRS, ≥9 point decrease in total HDRS score) the day after the TFD. 60% of 40 antidepressant-remitted depressed patients relapsed (300% mean HDRS increase) the day of the TFD, with return to remitted state the day after. While 80% of monoamine oxidase inhibitor- or fluvoxamine-treated patients relapsed, only 20% of desipramine-treated patients relapsed. Implications: Rapid depletion of plasma TRP transiently reverses antidepressant response in most remitted depressed patients suggesting that the antidepressant effects of some of these drugs may be more dependent on 5HT availability than that of others. Clinical characteristics and ultimate treatment response of symptomatic, drug free depressed patients in relation to the behavioral response to TD will be presented.

377.8

CHRONIC DESIPRAMINE TREATMENT ELEVATES EXTRACELLULAR NOREPINEPHRINE IN HIPPOCAMPUS WITHOUT ATTENUATING STRESS-INDUCED NOREPINEPHRINE EFFLUX. Elizabeth D. Abercrombie, *Dept. of Behavioral Neuroscience, University of Pittsburgh, Pittsburgh, PA 15260.*

We have observed that the stress-induced efflux of norepinephrine (NE) in hippocampus is significantly augmented in animals previously exposed to chronic stress [Nisenbaum et al., Soc. Neurosci. Abstr., 15 (1989), 413]. This effect may be relevant to the role of the locus coeruleus (LC) NE system in stress-induced behavioral disorders such as depression and antidepressants may thus act in part to dampen the transmission of information in that system. Therefore, the effect of treatment for 14 days with the antidepressant drug desipramine (DES; 10 mg/kg/day x 2, i.p.) was examined with regard to both resting and stress-induced efflux of NE in the hippocampus of rats using in vivo microdialysis. In DES treated animals, the basal level of NE in dialysates was 46 ± 5 pg/20 µl sample (corrected for probe recovery) whereas in control animals this value was 10 ± 1 pg/sample. Despite this difference, the magnitude of the increase in NE efflux in response to tail-shock stress was 59 ± 6% in DES treated animals vs. 61 ± 9% in control animals. The dose-response curve for inhibition of NE release by clonidine was shifted significantly to the right by chronic DES treatment over the range of doses tested (.025 to .25 mg/kg, i.p.). Thus, decreased function of inhibitory presynaptic α_2 adrenoceptors could permit continued responding of LC neurons to stress despite the elevated resting NE level. The above changes, along with associated downregulation of postsynaptic β adrenoceptors, may contribute to the antidepressant effects of chronic DES treatment by normalizing the overall level of NE transmission in a system hyperresponsive to activation by stress.

377.9

MEPACRINE BLOCKS DESIPRAMINE INDUCED BETA ADRENOCEPTOR DOWNREGULATION IN C6 GLIOMA CELLS. H. Manji, G. Chen*, I. Bitran*, F. Gusovsky, and W. Z. Potter*. Sec. Clin. Pharmacol., Clin. Neurosc. Branch, NIMH, and Lab of Bioorganic Chemistry, NIDDK, NIH, Bethesda, MD 20892.

Chronic treatment with a number of antidepressants (ADs) results in a downregulation/desensitization of rat cortical beta adrenoceptors (βARs). Although this effect has generally been attributed to an elevation of synaptic norepinephrine, the recent demonstration of similar βAR alterations in C6 glioma cells and human fibroblasts following in vitro incubation with desipramine (DMI), has also implicated direct postsynaptic mechanism(s). Mepacrine (MEP), a phospholipase A_2 (PLA2) inhibitor, has been shown to attenuate agonist and restraint stress induced βAR downregulation in C6 glioma cells and rat hypothalamus respectively. In order to investigate the possible role of PLA_2 in AD-induced βAR downregulation, C6 cells were incubated with DMI (10µM) alone, or in combination with MEP (10µM) for 5 days. DMI resulted in a 25% decrease in the density of βARs, and a similar reduction in isoproterenol (but not forskolin) stimulated cyclic AMP accumulation in intact C6 cells. MEP alone had no effect on βAR numbers or sensitivity but markedly attenuated both the DMI induced βAR downregulation and desensitization; these results suggest that arachidonic acid and/or its metabolites may be involved in DMI's effects. Preliminary results suggest that protein kinase C (PKC) is not involved in DMI's effects in C6 glioma cells.

377.10

CHARACTERIZATION OF THE EFFECT OF ANTIDEPRESSANTS IN THE TAIL SUSPENSION TEST USING CD-1 MICE. M.E. Nevins and P.M. Beardsley*, Dept. of CNS Diseases Research, G.D. Searle & Co., Skokie, IL 60077.

The tail suspension test is a new behavioral test for the identification of potential antidepressant drugs. It has been suggested that mouse strain is an important factor in sensitivity to antidepressants in this test. Therefore, the purpose of the present study was to characterize the response of the CD-1 mouse strain to the effects of various types of antidepressants in the tail suspension test. Groups of 15 mice were administered test drug s.c., and either 30 or 60 min later, were suspended from tape attached to their tails for a 6 min test session. The Itematic TST-1 (I.T.E.M.-Labo) was used to record the total time spent in immobility and the average power of the movements the mice made while suspended. The results demonstrate that using CD-1 mice the typical tricyclic antidepressants (i.e., imiprimine, desiprimine, etc.), the DA re-uptake inhibitor, bupropion, and the 5-HT re-uptake inhibitor, zimelidine, decrease immobility. However, the CD-1 mice appear to be very sensitive to the sedative effects of the antidepressants with high sedative liability, such as amitriptyline, mianserin and trazodone. These drugs tended to produce slight, not statistically significant decreases in immobility at low doses, and to increase immobility at higher doses. In conclusion, the CD-1 mouse appears to be sensitive to the antidepressant effects of several types of antidepressant drugs, and therefore, is useful (with the *caveat* that compounds with high sedative liability may not be detected) for the screening of potential antidepressant drugs in the tail suspension test.

377.11

NEUROENDOCRINE EFFECTS OF TANDOSPIRONE (SM-3997) IN HEALTHY MALE SUBJECTS. C. T. FISCHETTE, P. L. DELGADO, J. SEIBYL, J. H. KRYSTAL, G. R. HENINGER, D. S. CHARNEY. Dept. of Psychiatry, Yale Univ. Sch. of Med., New Haven, CT 06508 & Pfizer Pharmaceuticals, Inc. NY, NY 10017.

Tandospirone, a selective 5-HT_{1A} partial agonist, currently is being studied for its efficacy in depression. This study explores the neuroendocrine profile of tandospirone in fasting healthy subjects. Subjects received single doses of either placebo, 30, 40 or 50mg tandospirone at weekly intervals. At each testing session beginning at approximately 8:00 AM, blood was collected via an indwelling catheter at -20, -5, 30, 60, 90, 120, 150 and 240 minutes after oral drug administration for plasma levels of growth hormone (GH), prolactin and cortisol. In preliminary data (N=7) peak minus baseline GH concentrations were (mean ± S.D.): 0.1 ± 1.2ng/ml after placebo and 7.3 ± 7.1ng/ml ($p<.05$), 9.2 ± 7.6ng/ml ($p<.05$) and 7.7 ± 8.4ng/ml ($p<.09$) after 30, 40 and 50mg tandospirone, respectively. Cortisol levels peaked in a few subjects but no effect on prolactin was evident. Tandospirone exhibited rapid absorption and metabolism to 1-phenyl piperazine (1-PP), a common metabolite of this class of drugs which exhibits $alpha_2$ antagonist activity in animal studies. 1-PP levels were much higher than the parent compound. Increases in GH, but not prolactin, suggest differential regulation of these hormones by serotonergic and/or $alpha_2$ noradrenergic receptor systems.

377.12

COMPARISON OF THE EFFECTS OF MIANSERIN ENANTIOMERS AND METABOLITES ON A BEHAVIORAL SCREEN FOR ANTIDEPRESSANT ACTIVITY. G.J. Marek, T.H. Hand and L.S. Seiden. Dept. Pharm/Physiol.Sci., Univ. of Chicago, Chicago, IL 60637.

The behavioral effects of racemic mianserin, its (+) and (-) enantiomers, and its metabolites desmethylmianserin and 8-hydroxymianserin were evaluated on the differential-reinforcement-of-low-rate 72-sec (DRL 72-s) schedule, an operant behavioral screen known to be sensitive to and specific for the antidepressant properties of drugs. Racemic mianserin produced the antidepressant-like effect (increased reinforcement rate, decreased response rate) at 5 and 10 mg/kg. The mianserin enantiomers showed the antidepressant-like effect beginning at lower doses ((+)-mianserin: 0.6 mg/kg; (-)-mianserin: 2.5 mg/kg). The mianserin metabolites showed no clear dose-related effect at doses up to 10 mg/kg. It is concluded that the antidepressant-like effects of mianserin are due to the activity of the parent compound rather than to its metabolites, and that they may be primarily attributable to the (+) enantiomer. Given the known pharmacological profile of the mianserin enantiomers and metabolites, the present results suggest that the antidepressant-like effects of mianserin on DRL behavior are mediated through antagonist action at the 5-HT_2 receptor. This work supported by PHS MH-11191 and RSA-10562 (L. Seiden).

377.13

DOWN-REGULATION OF 5-HT2 RECEPTORS AND BEHAVIORAL RESPONSES FOLLOWING CHRONIC TREATMENT WITH ANTIDEPRESSANT DRUGS.

I. Lucki and S. Wieland. Depts. of Psychiatry and Pharmacology, University of Pennsylvania, Philadelphia, PA 19104.

Chronic administration of different types of antidepressant drugs reduce the number of 5-HT2 receptors and diminish the magnitude of behavioral responses mediated by 5-HT2 receptors. This experiment examined the ability of prior depletion of 5-HT content caused by the neurotoxin 5,7-DHT to prevent the effects of chronic treatment with two different types of antidepressant drugs: the tricyclic desipramine or the monoamine oxidase inhibitor phenelzine.

Rats were pretreated with 5,7-DHT (200 ug i.c.v. with 25 mg/kg desipramine) or vehicle 7 days prior to the initiation of chronic drug treatments. Different groups of rats were then injected for 14 days with either 0.9% NaCl, desipramine (10 mg/kg b.i.d.), or phenelzine (10 mg/kg once daily). On Day 16, 24 hours following the cessation of drug treatment, rats were injected with the 5-HT2 agonist DOB (1.0 mg/kg) and tested for the elicitation of head shaking behavior. In separate groups of drug-treated rats, the density of 5-HT2 receptors in the frontal cortex was measured.

Chronic treatment for 14 days with either desipramine or phenelzine reduced the number of head shakes elicited by the 5-HT2 agonist DOB. 5,7-DHT pretreatment diminished the ability of phenelzine to reduce the 5-HT2-mediated response but had no effect on the reduction of the response caused by desipramine. 5,7-DHT pretreatment attenuated the reduction of 5-HT2 receptors caused by chronic phenelzine treatment but did not alter the reduction of 5-HT2 receptors caused by chronic treatment with desipramine. Different mechanisms probably underlie the reduction of 5-HT2 receptor density and 5-HT2-mediated behavioral responses by these antidepressant drugs.

377.14

CHRONIC ADMINISTRATION OF PHENELZINE AND N^2-ACETYLPHENELZINE CAUSES ELEVATION OF WHOLE BRAIN AMINE NEUROTRANSMITTER LEVELS AND α_2 RECEPTOR DOWN REGULATION.

K.F.McKenna*, G.B.Baker, R.T.Coutts* and A.J.Greenshaw*. Neurochemical Research Unit. Dept. of Psychiatry, University of Alberta, Edmonton, Canada, T6G 2B7.

Phenelzine (PLZ), an irreversible nonselective monoamine oxidase (MAO) inhibitor, is widely used in psychiatry for the treatment of panic disorder (PD) and depression. PLZ has long been thought to be acetylated in humans but this is controversial. It has been shown that 1-acetyl-2-(2-phenylethyl)hydrazine [N^2-acetylphenelzine (N^2AcPLZ)] is a metabolite of PLZ in rat brain and blood. We have investigated the effects of chronic 28 d administration of PLZ (0.05 mmol/kg/d) and N^2AcPLZ (0.10 mmol/kg/d) on α_2 adrenoreceptor function using a behavioural test on days 21 and 22. After administration of physiological saline or the α_2 agonist clonidine (0.05 mg/kg), rats treated with PLZ and N^2AcPLZ displayed a significant attenuation of the suppressant effects of clonidine on spontaneous locomotor activity compared to controls, indicating down regulation of α_2 receptors. This finding is of interest as α_2 receptor supersensitivity is hypothesized in patients with PD and PLZ has antipanic efficacy. By day 28 both PLZ and N^2AcPLZ had produced greater then 90% inhibition of MAO-A and -B in liver and heart. Both drugs induced significant elevation of whole brain noradrenaline, 5-hydroxytryptamine (5-HT), and dopamine levels compared to controls. The acid metabolites of dopamine and 5-HT [homovanillic acid, 3,4-dihydroxyphenylacetic acid and 5-hydroxyindole-3-acetic acid (5-HIAA)] were significantly reduced in drug treated animals. There were no significant differences between the effects of PLZ and N^2AcPLZ in these studies (except in the case of 5-HIAA), suggesting that N^2AcPLZ is a potent nonselective MAO inhibitor which can downregulate α_2 receptors.

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377.15

G-PROTEINS IN RAT BRAIN: EFFECTS OF LITHIUM.

M.B. Williams, F.H. Wagner*, X. Gu, M.S. Baird* and R.S. Jope. Depts. Pharmacology and Psychiatry, University of Alabama, Birmingham, AL 35294.

Lithium is the primary treatment for bipolar affective disorder, but its mechanism of action is unknown. Recent evidence suggests that a primary site of action of lithium may be the G-proteins associated with second messenger systems in the brain. Therefore, we measured the effects of chronic treatment of rats with lithium on the ADP-ribosylation of G-proteins and on the G-protein mRNA levels.

ADP-Ribosylation of G-proteins was measured in tissue from rat hippocampus and cortex. Three processes were measured, ADP-ribosylation induced by pertussis toxin, cholera toxin, and the endogenous ARF (ADP ribosylation factor). ADP-Ribosylation was measured by incubation of membranes (for pertussis or cholera toxin) or homogenates (for ARF) with ^{32}P-NAD, ATP, thymidine, ATP, triton-X-100, and guanine nucleotides followed by electrophoresis on 10% polyacrylamide gels, autoradiography, and quantitative densitometric scans. With pertussis toxin, APD-ribosylation was maximal with GDP and was reduced by a stable analog of GTP, and was limited to proteins of approximately 40 kD. With cholera toxin and ARF, ADP-ribosylation was maximal with a stable analog of GTP and several proteins were labelled.

Total RNA was isolated from rat hippocampus and cortex by acid guanidinium thiocyanate-phenol-chloroform extraction and mRNA was analyzed by Northern blot hybridizations using G_s, G_o, G_{i1}, G_{i2}, and G_{i3} cDNA clones generously provided by Dr. R. Reed.

Rats were treated with dietary LiCl for 4 weeks using a protocol which causes no weight loss and produces plasma lithium levels of approximately 0.8 mM. For each assay, lithium-treated and control rats were run in parallel and the results quantitatively compared. The results of these studies should indicate whether chronic lithium treatment affects the mRNA levels for G-proteins or the G-proteins available for ADP-ribosylation by any of the three methods studied.

377.16

LITHIUM ATTENUATES ADENYLATE CYCLASE ACTIVITY IN INTACT RAT BRAIN: AN IN VIVO MICRODIALYSIS STUDY.

M. I. Masana, J. A. Bitran*, J. K. Hsiao* and W. Z. Potter*. Sec. Clin. Pharmacol., Clin. Neurosc. Branch, NIMH, Bethesda, MD 20892

The effects of chronic and acute lithium treatment on adenylate cyclase activity in intact rat brain were examined using *in vivo* microdialysis. Basal extracellular cyclic AMP was measurable in dialysate when the phosphodiesterase inhibitor, rolipram, was present (0.52 ± 0.06 fmol/min, n=14) and increased in a dose dependent manner after agonists were added to the perfusate. The increase in cyclic AMP in brain extracellular fluid after stimulation with 100 µM norepinephrine was blocked by addition of 1 µM of the ß-blocker, propranolol ($p<0.025$). Chronic lithium treatment (8 weeks, serum level 0.7 mEq) modestly increased basal brain extracellular fluid cAMP levels, while significantly blunting the cyclic AMP response to stimulation with 100 µM norepinephrine (57.9% increase after lithium versus 170.6% increase in controls, $p<0.005$). Acute infusion of lithium also modestly inhibited the cyclic AMP response to norepinephrine stimulation (99.7% increase). The effects of lithium treatment on different components of the adenylate cyclase transduction system (forskolin and cholera toxin stimulated cyclic AMP increase) were also assessed.

377.17

INOSITOL PHOSPHATES IN RAT BRAIN REGIONS: EFFECTS OF LITHIUM AND SEIZURES.

R.S. Jope, R.E. Smith* and R.A. MacQuarrie. Dept. of Psychiatry, University of Alabama, Birmingham, AL 35294 and University of Missouri, Kansas City, MO 64110.

Anion exchange chromatography coupled with detection by chemical suppression was used to measure inositol phosphates in rat brain regions after sacrifice by microwave irradiation. Coadministration of acute lithium and pilocarpine induced seizures which were accompanied with large increases of $InsP_3$ and $InsP_2$ in the hippocampus and cortex.

Chronic dietary LiCl (4 weeks) produced brain Li levels of 0.60-0.97 mmole/kg and resulted in 74 to 87% depletions of $InsP_3$ and $InsP_2$. Stimulation of seizures with pilocarpine only slightly increased $InsP_3$ but significantly elevated $InsP_2$.

In contrast to inositol phosphates, chronic Li did not alter the concentration of cyclic AMP nor did it reduce the elevation induced by seizures.

These results demonstrate directly that chronic lithium treatment severely reduces phosphoinositide metabolism in rat brain regions, but the system is still capable of responding to a stimulus. Furthermore, the effects of chronic lithium were much more evident on inositol phosphates than on cyclic AMP.

377.18

LITHIUM, CALCIUM, AND LOCOMOTOR ACTIVITY IN SYRIAN HAMSTERS.

K.E. Greene*, H. Klemfuss, and D.F. Kripke*. Depts. of Neurosciences and Psychiatry, University of California, San Diego, and VA Medical Center, San Diego, CA 92093.

Lithium (Li+) is an effective antidepressant and anti-manic agent, but its mechanism of action is not well understood. Lithium's primary action may be to compete with endogenous cations for binding sites. This study tested interactions of dietary calcium (Ca++) and lithium on plasma Ca++ concentration, locomotor activity, and toxicity in 30 male Syrian hamsters.

A diet high in calcium (3% $CaCl_2$) significantly increased total running wheel locomotor activity by 65% compared to a low calcium diet (0.1% $CaCl_2$). Addition of lithium (0.4% Li_2CO_3) to either low or high Ca++ diets, producing plasma Li+ concentrations ranging from 0.6 to 2.0 mM, significantly increased both plasma Ca++ and locomotor activity. Overall activity levels were significantly correlated with plasma Ca++ levels. While there was no difference in toxicity between the low and high Ca++ diets, calcium protected against some of lithium's toxic side effects.

Although central homeostatic mechanisms protect the brain from changes in peripheral calcium, we found that a modest change in plasma calcium concentration can have clear effects on CNS-controlled behavior. The data also support a stimulatory action of lithium on locomotor activity, one that may be mediated through calcium.

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377.19

ALTERED FENFLURAMINE- STIMULATED PROLACTIN RELEASE DURING TREATMENT OF OBSESSIVE COMPULSIVE PATIENTS. W.A. Hewlett*. Dept. of Psychiatry, Stanford University School of Medicine. Stanford.CA.94305.

OCD is a psychiatric disorder which may involve alterations in serotonin functioning. In order to assess serotonergic functioning in OCD patients fenfluramine-stimulated prolactin release was measured in both a medication free condition and during treatment with either clomipramine, clonazepam, clonidine, or diphenhydramine. Two baseline prolactin samples were drawn 30 minutes apart prior to administration of a 60 mg oral dose of fenfluramine. Fenfluramine-stimulated prolactin samples were then drawn hourly over the next five hours. Treatment with clomipramine significantly elevated baseline prolactin levels as compared to the untreated condition. Female patients showed significantly greater elevations in fenfluramine-stimulated prolactin release in both treated and untreated conditions. Fenfluramine-stimulated prolactin release was significantly greater under conditions of clonazepam and clomipramine treatment than that seen during diphenhydramine treatment. These results are consistent with altered serotonergic functioning during serotonergic treatment of OCD.

377.20

NORADRENERGIC DYSFUNCTION IN PANIC DISORDER. D.S. Charney, S.W. Woods, J.H. Krystal, L.M. Nagy, G.R. Heninger. Psychiatry Service, West Haven VA Medical Center, West Haven, CT. 06516.

Abnormal regulation of brain noradrenergic neurons may be involved in the pathophysiology of anxiety disorders. This investigation evaluated noradrenergic function in panic disorder by evaluating responses to intravenous yohimbine and clonidine in the same panic disorder patients and healthy subjects. **METHODS**: Thirty-eight patients and 15 healthy subjects (HS) participated in the study. Infusions of clonidine (0.2 µg/kg), yohimbine hydrochloride (0.4 mg/kg), or saline, over 10 min. was given during a series of 3 test days. Before drug administration, and at intervals thereafter, behavioral ratings, blood pressure, heart rate, and plasma samples for MHPG and growth hormone were obtained. Each patient was assessed to determine whether a panic attack occurred. **RESULTS**: Yohimbine produced panic attacks in 63% of the patients. Patients who experienced yohimbine induced panic attacks (YPA) had greater increases in plasma MHPG than the patients who did not (NPA) and the HS. The YPA, but not the NPA, patients had a blunted growth hormone rise to clonidine compared to the HS. Clonidine produced significant decreases in plasma MHPG and anxiety symptoms in the YPA patients, but not in the NPA and HS. **CONCLUSIONS:** Abnormal yohimbine induced increases in plasma MHPG, blunted growth hormone responses to clonidine, and significant clonidine induced decreases in plasma MHPG and anxiety symptoms are restricted to YPA patients. These patients may represent a subgroup of panic disorder patients with noradrenergic neuronal dysfunction, specific clinical characteristics and treatment response.

377.21

LITHIUM AUGMENTATION IN FLUVOXAMINE-REFRACTORY OBSESSIVE COMPULSIVE DISORDER. W.K. Goodman, M.D., C.J. McDougle, M.D., L.H. Price, M.D., D.S. Charney, M.D., G.R. Heninger, M.D., Yale Univ. Dept. of Psychiatry, 34 Park St., New Haven, CT 06519.

These double-blind placebo-controlled studies examined the efficacy of adding lithium carbonate, which may augment serotonin (5-HT) function, to an ongoing treatment trial of fluvoxamine (FVX) in 30 patients with primary OCD who had failed to respond to FVX. Methods: Study 1: 20 patients (4 inpatients, 16 outpatients) with OCD (DSM-III-R) were randomized to 2 weeks of treatment with active (N=11) or placebo (N=9) lithium augmentation of ongoing FVX treatment. Study 2: An additional 10 outpatients with OCD were randomized to 4 weeks of treatment with active (N=5) or placebo (N=5) lithium augmentation of ongoing FVX treatment. Outcome was assessed with Y-BOCS scores before and after the addition of lithium to FVX. Results: Study 1: Two of 11 (18%) patients met criteria for a meaningful clinical response (1 marked, 1 partial) to active lithium. No patients responded to placebo. Active lithium augmentation of FVX produced a statistically significant improvement in scores on the Y-BOCS (-4.0±5.2, $p<.03$, paired t-test, two-tailed). There was no significant change in severity of OCD symptoms in the placebo lithium-treated group. There were significant between-group differences as measured by the Y-BOCS ($p<.04$, student's t-test). An analysis of all patients treated subsequently with 3-5 weeks of open active lithium augmentation (N=16) found a statistically significant improvement in Y-BOCS scores (-3.1±2.5, $p<.003$), 5/16 (31%) patients showed a qualitative response (1 marked, 4 partial). Study 2: No patients demonstrated a response during the 4-week controlled trial of lithium augmentation of FVX. In addition, there was no significant change in scores on the Y-BOCS. Combined Analysis: For all patients receiving 4 weeks of open active lithium augmentation of FVX (N=20), no significant change in Y-BOCS scores was found (-1.5±5.2, n.s.). Only 4/20 (20%) of these patients showed a response (1 marked, 3 partial). Conclusion: These double-blind placebo-controlled trials suggest that lithium augmentation is not an effective strategy in most OCD patients. The different rate of response to this treatment strategy between patients with OCD and depression suggests that pathophysiological differences may exist between these two disorders.

LEARNING AND MEMORY: PHYSIOLOGY V

378.1

ORBICULARIS OCULI AND EXTRAOCULAR MUSCLE ACTIVITY DURING UNCONDITIONED AND CONDITIONED EYEBLINKS IN THE RABBIT. N.E. Berthier, J.W. Moore, Department of Psychology, University of Massachusetts, Amherst, MA 01003.

The activity of the retractor bulbi (RB), superior rectus (SR), and orbicularis oculi (OO) muscles were recorded in awake animals using chronically and acutely implanted teflon coated microwires. Eyelid and nictitating membrane positions were measured with potentiometers. All three muscles showed a two component response to trigeminal nerve stimulation, air puff, and periorbital electrostimulation. With periorbital electrostimulation the latencies of the two responses were about 5 and 10 ms. Crosscorrelegrams of EMG activity taken from conditioning trials showed less than 5 ms shift in the peak correlations. Examination of individual conditioning trials showed that the time course of EMG activity in the RB, SR, and OO muscles was highly correlated. Two differences in EMG activity were seen between muscles: (1) The OO showed tonic activity while the RB and SR did not. (2) The SR showed small EMG responses relative to the RB and OO. Given the high correlation between the activity of extraocular and OO activity we conclude that muscle activity during blinks is the result of a single premotor system. (Supported by AFOSR 89-0391 and BNS 88-10624).

378.2

GENOTYPE AND REGION DEPENDENT BRAIN DNA SYNTHESIS DURING HABITUATION TO SPATIAL NOVELTY IN THE ALBINO RAT. A.G. Sadile*, A. Cerbone*, C. Lamberti-D'Mello**, T.Menna, C. Buono, F. Rafti & A. Giuditta**. IIGB, CNR, Naples; *Dipt. Fisiol.Um. & Funz.Biol.Integr. "F.Bottazzi"; and **Fisiol.Gen. & Amb., Univ. Naples Federico II, Naples, I.

To investigate the role of brain DNA synthesis in non associative learning, adult male albino rats of a random-bred Sprague-Dawley stock (NRB), and of the Naples High (NHE) and Low-Excitability (NLE) strains, selectively bred for divergent activity in a novel environment (Sadile et al., Soc.Neurosci. 9:643, 1983) were used. Three groups of rats from each strain were exposed or re-exposed one day later, or unexposed to a L-maze. The frequency of corner crossings and rearings were monitored during a 15min-test. Rats received an intraperitoneal injection of 3H-thymidine 15 min before test trial 1 or 2. Brain DNA synthesis was measured in ex-vivo homogenates of neocortex, hippocampus, neostriata, midbrain, hypothalamus and cerebellum by the incorporation of 3H-thymidine into DNA after a 30min pulse. Both NLE/NHE rats had a higher basal DNA synthesis in the hippocampus and neostriata. Upon test trial 1, but not upon re-test, there was a significant decrease in DNA synthesis in the neocortex and hippocampus of both strains, and in the hypothalamus, midbrain and cerebellum of NLE only. The decrease was lower in NHE than in NLE rats. The results support the proposed involvement of brain DNA in information processing and storage.

378.3

IMMUNITY FROM NICOTINE-INDUCED CONDITIONED FOOD AVERSION IN MICE. N.E. Kinney. Dept. of Psychology, SE Missouri State Univ., Cape Girardeau, MO 63701.

Mice (Mus musculus) demonstrated dose-dependent conditioned food aversion (CFA) when novel food (almond) consumption was paired with a subcutaneous injection of (-) nicotine tartrate (0.2 - 1.6 mg/kg). Controls showed no aversion (but rather a significant increase in consumption) at 0.2 mg/kg, and complete aversion at 1.6 mg/kg. This contrasts with reported aversion to nicotine at 0.1 mg/kg in rats. Immunity to nicotine-induced CFA was demonstrated by bilaterally olfactory nerve-sectioned mice on day 7 post-surgery (while anosmic). This protection was lost by day 15 post-surgery.

The novel food effect shown by controls was not observed in nerve-sectioned mice. These findings are in agreement with earlier work using tilted-rotary motion to produce malaise. The loss of CFA in anosmic animals (and its appearance following olfactory nerve regeneration) for both motion-induced and nicotine-induced malaise strengthens support for a dominant role of olfaction in the formation of conditioned flavor aversions.

378.4

PLASTICITY IN THE REINFORCEMENT SYSTEM OF INFANT RATS. R.M. Sullivan and D.A. Wilson. Developmental Psychobiology Laboratory, Dept. Psychology, University of Oklahoma, Norman, OK

Newborn rat pups learn to approach odors paired with tactile stimulation which mimics stimulation during maternal care. This learned behavioral preference is associated with a modified olfactory bulb neural response to that odor. Odor-stroking pairings in pups after PN8-10, however, do not produce these learned neurobehavioral responses, suggesting a sensitive period in early olfactory learning. The present study examined the nature of this age sensitivity.

Pups were trained on PN5, PN12 or PN20 in an olfactory conditioning paradigm employing either STROKING, intra-oral MILK infusions, LOW intensity foot shock (0.5 mA) or HIGH intensity foot shock (1.5 mA) as the UCS. Training consisted of a 10 min CS/UCS pairing, or randomized UCS - CS presentation. Pups were tested for neurobehavioral responses to the odor CS 24 hr later. The results suggested that while STROKING was only effective at PN5, pups could learn odor preferences at all ages if trained with MILK as a UCS. HIGH shock produced odor aversions at all ages, while LOW shock produced odor preferences at PN6 and aversions at older ages.

These results suggest that sensitive periods in early olfactory learning may reflect ontogenetic changes in reinforcement pathways, rather than in the olfactory system itself.

378.5

CHANGES IN MEMORY FUNCTION ACROSS THE MENSTRUAL CYCLE S.M. Phillips and B.B. Sherwin. Department of Psychology, McGill University, Montreal, P.Q., Canada, H3A 1B1

Thirty healthy cycling women (mean age 23.6 years) underwent cognitive testing during the menstrual (M) and midluteal (ML) phases of the menstrual cycle. Radio-immunoassay was performed on blood samples drawn at both test times in order to measure plasma estradiol (E2) and progesterone (P) levels. Based on hormone levels, menstrual cycle phase was confirmed for 25 subjects.

In this confirmed sample, higher scores on a visual memory (30-minute delayed recall) task occurred in the ML relative to M phase ($p<.01$), coincident with higher levels of plasma E2 ($p<.001$) and P ($p<.001$). No significant differences across the menstrual phases were found on verbal memory (immediate or delayed recall), visual memory (immediate recall), digit span, or paired-associate learning. There was, however, a significant positive correlation within the ML phase between plasma P and verbal memory scores (immediate recall, $p<.04$; delayed recall, $p<.03$); whereas plasma E2 was positively correlated with paired-associate learning scores ($p<.03$).

Results indicate that mild variations in memory function occur in some women across the menstrual cycle in association with changes in plasma E2 and P levels.

378.6

MAPPING OF AUDITORY MEMORY WITH 2-DEOXYGLUCOSE: LATERALIZED CONDITIONING EFFECTS IN SPLIT-BRAIN RATS AND GERBILS. F. Gonzalez-Lima, H. Scheich, and A.R. McIntosh. Dept Med Anat, Texas A&M Univ, College Station, TX 77843; Inst Zool, Tech Univ, Darmstadt, W Germany.

The 2-DG method was used to map memory-related activity in twelve split-brain rats and gerbils. Training started 2 weeks after surgery and consisted of unilateral presentations of sound and foot shock in paired (one side) and unpaired (other side) sessions. The next day, subjects were tested for conditioned suppression of drinking, injected with 2-DG, and stimulated binaurally with sound. Differences in 2-DG uptake between the two sides of the brain were quantified. The conditioned hemisphere (side contralateral to paired stimuli) showed striking enhancements of uptake in forebrain structures, including hippocampus, subiculum, accumbens, entorhinal and perirhinal cortex, amygdala, and fundus striati. The largest increase (70%) was in the CA2 region of the hippocampus. The findings revealed for the first time a direct, within subjects, comparison of the cerebral representation of auditory memory. (Supported by NIMH grant RO1-MH43353).

378.7

NEURAL SUBSTRATES FOR DISCRIMINATIVE OPERANT CONDITIONING VISUALIZED USING 2-DEOXYGLUCOSE AUTORADIOGRAPHY. M. Garrosa, F. Gonzalez-Lima and F.J. Helmstetter. Dept Med Anat, Texas A&M Univ, College Station, TX 77843; Univ Valladolid, Spain.

2-DG techniques applied to rats trained in four groups were used to map the neural respresentation of the behavioral components involved in discriminative operant conditiong. 1) Rats trained to bar press for food during a sound discriminative operant conditioning (sound → operant CR → reward); 2) Rats without bars yoked to those in Group 1 (sound → reward); 3) Rats trained to bar press for food, independently of sound, nondiscriminative operant conditioning (random sound, operant CR → reward); 4) Rats without bars yoked to those in Group 3 (random sound and reward). Group 1 rats (disc.) compared to 3 (nondisc.) showed 2-DG changes in accumbens, ventral cochlear and ventroposteromedial thalamic nuclei. Structures with the same 2-DG uptake in the operant groups (1=3) but different than the nonoperant groups (2,4) included frontal cortex, paratenial and reticular thalamic nuclei, medial geniculate and cerebellar vermis. Common changes in the conditioned groups (1=2=3) but different than the random group (4) included the caudal caudatoputamen, posterior parietal cortex, dorsal cochlear nucleus, and cerebellar flocculus. These changes are the first demonstration of brain metabolic alterations related to a learned discriminative response to sound. (Supported by NIMH grant RO1 MH 43353)

378.8

PHYSIOLOGICAL PROPERTIES OF MEDIAL SEPTAL NEURONS (MSNs) IN UNANESTHETIZED RATS. J.E. Sweeney, M.H. Bassant* and Y. Lamour, INSERM U 161, 75014 Paris France.

Rhythmically bursting neurons (RBNs) of the medial septum may trigger one type of hippocampal theta rhythm that correlates to behavior in the rat. Since the activity of RBNs varies with different anaesthetics, we have chosen to characterize their properties in unanesthetized rats habituated to a painless restraint.

In 355 MSNs (recorded from 12 rats), 59 neurons (17%) exhibited a rhythmic bursting activity. This percentage is clearly different from the 45% of bursting MSNs recorded in urethane-anesthetized rats. The bursting activity was also of a significantly higher frequency than with urethane (5.8 ± 0.1 vs. 3.9 ± 0.1 bursts/sec). The majority of bursting neurons were recorded during EEG arousal (with a theta). Nevertheless, 2 bursting neurons were recorded in "drowsy" states (in the absence of a theta) and 10 during paradoxical sleep (with a frequency of 6.2 ± 0.2 bursts/sec). Tactile or electrical stimulation of the reticular formation induced EEG arousal and rhythmicity in 13 of 58 previously non-bursting neurons that had a high (>15 spikes/sec) spontaneous activity.

Clearly, properties of rhythmically bursting neurons differ between unanesthetized and urethane-treated rats. These results also suggest that there may be discrete pools of MSNs--a small number that maintain rhythmicity regardless of the state of arousal and a reserve population of rapidly firing cells that can be recruited by an arousing stimulus to fire rhythmically.

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378.9

THE CONTRIBUTION OF THE MEDIAL SEPTAL AREA TO ENTORHINAL CORTICAL AND SUBICULAR SINGLE-UNIT ACTIVITY. S. J. Y. Mizumori and K. E. Ward*, Dept. Psych., Univ. Utah, Salt Lake City, UT 84112

Reversible inactivation of the medial septum temporarily disrupts spatially selective discharge of hippocampal hilar/CA3 (and not CA1) cells, reduces movement-sensitivity of stratum granulosum (SG) units, and produces a spatial working memory impairment in rats (Mizumori et al., *J. Neurosci.*, 1989). Recently, we examined the possibility that septal inactivation produced these effects by functionally isolating hippocampus from its primary input (entorhinal cortex) and/or output (subiculum) structures.

Spontaneous single unit activity was monitored in hippocampus (CA1: n=16; FD: n=16), dorsal subiculum (n=20), and medial entorhinal cortex (n=13) before, during, and after septal inactivation in 10 Nembutal-anesthetized F-344 rats. Baseline activity was recorded for 10 min, lidocaine (0.5 µl; 2% solution) was injected into the medial septal area, then unit activity was monitored for at least an additional 20 min. Similar to previous results, few (6%) CA1 complex-spike cells and 50% of SG and hilar/CA3 cells showed altered discharge patterns following lidocaine treatment. A small percentage of subiculum (10%) and entorhinal (15%) cells responded by either increasing or decreasing firing. Ongoing experiments with freely behaving animals thus far confirm this pattern of responses. These data suggest that septal inactivation-induced alterations in subcortical, rather than entorhinal, afferents underlie the hippocampal unit effects. Furthermore, the behavioral impairment described earlier may have resulted from a disruption of hippocampal, and not subicular, unit activity. [Supported by BRSG Grant S07 RR07092]

378.10

MEDIAL SEPTAL AREA CODES MNEMONIC COMPONENTS OF A WORKING MEMORY TASK. B. Givens and D.S. Olton. Dept. Psychology. Johns Hopkins University. Baltimore, MD 21218

The contribution of the medial septal area (MSA) to working memory processes was investigated in rats performing a delayed continuous conditional discrimination (CCD) operant task. Single neurons in the medial septum, lateral septum and CA1 hippocampus were analyzed for changes in activity that correlated with components of the task. In addition, MSA neural output was altered by intraseptal microinfusions, and changes in operant behavior and hippocampal EEG were recorded. Single unit analysis revealed that although some CA1 complex spike cells fired differentially to the task components, most hippocampal neurons showed little event-related firing. Conversely, most septal cells fired differentially to the stimulus/response contingencies. Microinfusion of tetracaine or muscimol into the MSA suppressed hippocampal theta and decreased choice accuracy in the CCD task. These changes returned to baseline more rapidly following tetracaine (25 min) than muscimol (60 min), and at shorter interstimulus delay intervals. The changes in EEG and choice accuracy were correlated most strongly at the longest (20 sec) delay. Taken together, these data support a role for the MSA in the neural coding of non-spatial information important for working memory in rats. Supported by NRSA # NS8616.

378.11

CHOLINERGIC ACTIVATION OF MEDIAL SEPTAL AREA CAN RESTORE WORKING MEMORY IN OLD RATS AND IN SCOPOLAMINE-TREATED YOUNG RATS. A. L. Markowska, B. Givens, and D.S. Olton, Dept. of Psychology, The Johns Hopkins University, Baltimore, MD 21218.

Functional deterioration in the cholinergic system with age may be partly responsible for age-related memory deficits. Therefore, activation of cholinergic neurons in the basal forebrain may alleviate these memory impairments. In the present study, naive young (4 mo), scopolamine-treated young (4 mo), or naive old (24 mo) rats were microinfused into the medial septal area (MSA) with cholinergic receptor agonists (carbachol, oxotremorine), a GABA receptor antagonist (bicuculine), or saline. Working memory, as assessed by choice accuracy in a spatial alternation task, was tested before and after infusion, then compared to baseline performance during saline and non-infusion trials. Preliminary data indicate that cholinergic agonists infused directly into MSA can partially reverse scopolamine-induced deficits in young rats and may also improve performance in the aged rats in this task. These data suggest that cholinergic drugs may act directly on basal forebrain cholinergic neurons for their mnemonic effects. Hippocampal EEG recordings before and after microinfusion are currently being analyzed for electrophysiological correlates of the behavioral data. Supported by NRSA # NS8616.

378.12

A CORRELATE FOR OLFACTORY LEARNING IN AN IDENTIFIED NEURON IN THE HONEY BEE BRAIN. J. Mauelshagen* and R. Menzel. Institut für Neurobiologie, Freie Universität Berlin, Königin-Luise-Str.28/30, D-1000 Berlin 33, FRG.

A single large neuron extrinsic to the pedunculus of each mushroom body, identified by intracellular marking, responds to a variety of sensory inputs with excitation (olfactory, mechanical, taste, vision). Recording experiments were performed under conditions, which in behavioral experiments lead to sensitization and conditioning of olfactory stimuli (Menzel, R. in Olton and Kesner (eds) Neurobiology of comparative cognition, Erlbaum 1990). Compared to the control group, sensitization results in a significant frequency increase during the first 100 msec interval of the response. In contrast, conditioning results in a significant frequency decrease during the third and fourth interval of the response. This suggests different processes involved in coding the olfactory information depending on the temporal order of the CS and US. Further experiments have to ensure that the observed response modulations are specific effects of non-associative and associative memory processing.

378.13

Single Cell Specificity of Molecular Changes During Memory Storage. **D L. McPhie, L.D. Matzel, J.L. Olds, A.M. Kuzirian* and D.L. Alkon, LMCN, NINDS, NIH, Bethesda MD, 20892.**

***Hermissenda crassicornis* exhibits a learning-specific increase in Protein Kinase C that is localized to individual types of nerve cells as assessed by 3H-phorbol 12,13-dibutyrate ([3H]-PDBU) emulsion autoradiography in 4µm epon sections of circumesophageal ganglion. The increase is anatomically localized to neurons previously shown to be critically involved in the storage of a classically conditioned response which has been shown to undergo specific biochemical, electrophysiological and morphological modifications. The present study confirms and extends these previous results while implicating anatomically-localized increases in PKC to learning-specific molecular processes.**

The circumesophageal ganglion of animals in three treatments (Paired, Random and Naive) were incubated with 3H-PDBU and then processed for histology and subsequent computerized image analysis. Paired, but not Random or Naive, animals showed a significant increase in 3H-PDBU binding in the medial and intermediate B photoreceptors and in cells of the optic ganglion(17%, 10%, and 16% respectively. $p<.01$, n=6 per group, planned orthagonal contrast). Such increased binding of [3H]-PDBU in *Hermissenda* represents to our knowledge the first conclusive evidence for neuronal specificity of memory-specific changes in the distribution of this regulatory enzyme (Olds et al Science, 1989).

378.14

GABA-INDUCED PROTEIN KINASE ACTIVATION IS ENHANCED WHEN PAIRED WITH POST-SYNAPTIC DEPOLARIZATION.
L.D. Matzel and D.L. Alkon
Laboratory of Molecular and Cellular Neurobiology, NINDS-NIH

The characteristic inhibitory influence of vestibular hair cells on the B photoreceptors of the *Hermissenda*'s eye is mediated by a GABAergic synapse. Hair cell impulses (like that which accompany rotation) as well as exogenous GABA (12.5 μM) application activate a primary Cl^- conductance that is accompanied by a decrease in membrane resistance. Activation of a second, slower K^+ conductance, characterized by an increase in resistance is also observed 60-90 sec after GABA application. If the Cl^- channel blocker NPPB (10 μM) was added to the extracellular bath, this increase in resistance was observed within 10 sec of GABA application, suggesting that the second conductance is normally masked by the faster Cl^- conductance. Moreover, if the B cell was depolarized 20-40 mV by positive current injection (analogous to that which occurs in response to light), the magnitude of the GABA-induced increase in resistance was dramatically enhanced. This latter effect was not observed if Ca^{2+} was removed from the extracellular bath or if the protein kinase inhibitor H7 (100 μM) was added to the bath, suggesting that the activity of a GABA-activated protein kinase was enhanced by depolarization-induced intracellular Ca^{2+} elevation in the post-synaptic neuron. These results suggest a mechanism underlying the temporal dependence of stimulus pairings in classical conditioning, and in particular, the biophysical changes in single neurons which accompany light-rotation pairings in *Hermissenda*.

378.15

ACTIVATION OF Ca^{++}-DEPENDENT PROTEIN KINASE ALTERS BIOPHYSICAL RESPONSES OF ISOLATED *HERMISSENDA* PHOTORECEPTORS WHEN PAIRED WITH INTRACELLULAR Ca^{++} ELEVATION

I. Lederhendler, L. Matzel, P. Huddie, and D.L. Alkon. Laboratory of Molecular and Cellular Neurobiology, NIH-NINDS.

Following classical conditioning, *Hermissenda* Type B photoreceptors show increased input resistance and light responses. The protein kinase activator, phorbol ester (PDBU), has been shown to reduce outward K^+ currents that underlie this increased excitability. Ca^{++}-dependent activation of protein kinase C mediates long-term reduction of K^+ currents and also produces dramatic outgrowths from the soma of isolated photoreceptors. We studied the relationship of this morphological change to biophysical properties of Type B cells with current and voltage clamp, using specific conditions which produce outgrowth activity. In these isolated photoreceptors only pairing light with PDBU, but not light alone, nor light paired with an inactive phorbol ester, increased the light response and input resistance (ANOVA, $N=18$, $p<.01$). These changes could not be duplicated when Ca^{++} was absent from the bath. Furthermore, single-electrode voltage clamped photoreceptors showed reduced Ca^{++}-dependent K^+ currents when PDBU was present and Ca^{++} influx was increased by depolarizing voltage steps equivalent in magnitude to light stimulation used in the current clamp experiments. These results suggest that certain key biophysical and morphological changes in the Type B soma may be expressions of the same conditioning experience.

NEUROETHOLOGY: MAMMALS, REPTILES, AMPHIBIANS

379.1

RESPONSE OF TEMPORAL LOBE NEURONS TO SOCIAL STIMULI IN MACACA ARCTOIDES . L.A. Brothers, B.D. Ring* and A.S. Kling . UCLA/Sepulveda VA Medical Center, Sepulveda CA 91343

Accurate evaluation of social signals is essential to group-living primates; however, neural processing of the full range of social stimuli remains little understood.

We used laser disks containing 50,400 frames of moving pictures and vocalizations of macaques recorded in natural settings to deliver 2s clips of a broad array of body parts, movements, expressive behavior, and social interactions. Recordings of single unit activity in the region of the right amygdala and neighboring cortical areas were obtained in an alert stumptail macaque.

Of 275 isolated units, 21 were selectively responsive to a subset of the presented stimuli. Of these, 2 responded to one or a few specific pictures only. The remainder responded to a heterogeneous subset of the stimuli or were selective for a single feature common to a number of stimuli. These results suggest that anterior temporal neurons of the macaque brain participate in ensembles coding features of the social environment.

Supported by the Dept. of Veterans Affairs.

379.2

SPATIAL TUNING IN MUSTACHE BAT AUDITORY NEURONS WITHIN THE CONTEXT OF ECHOLOCATION. Z.M.Fuzessery. Dept. of Psychology and Zoology, Univ. of Wyoming, Laramie, WY 82071.

Echolocating animals have the potential to modify both their acoustic emitters and receivers to optimize signal analysis. This study examines how spatial processing in the mustache bat might be optimized. It combines data on the directionality of the external ears and the radiation pattern of the echolocation pulse (Hartley and Suthers, 1990) to determine the distribution of acoustic energy in the frontal sound field of the mustache bat while it echolocates. One finding is that intensity level is almost constant across the center of the sound field, from 40° to either side of the midsagittal plane. This is not the case when only ear directionality is considered. In addition, energy drops away more rapidly at locations lateral to 40°. This suggests that the echolocation system may stabilize one stimulus parameter, intensity level, at the center of the sound field to allow for a less ambiguous analysis of target location, as well as other features of the scanned object. This, for example, would allow the bat to resolve interaural intensity differences independent of absolute intensity level. Moreover, binaurally facilitated neurons that exhibit a broad spatial tuning when only ear directionality is considered show a sharper selectivity when evaluated within the context of echo-location. In addition to maintaining an almost constant intensity level across the center of the sound field, the mustache bat also "intensity compensates" (Henson et al., 1985) to maintain the echo at an absolute intensity level. These findings suggest that this echolocation system may be optimized to reduce the ubiquitious problem of differentiating stimulus quality and quantity. NS-13276, NS-21286.

379.3

REPRESENTATION OF TARGET ELEVATION IN UNIFIED IMAGES PERCEIVED BY FM ECHOLOCATING BATS. J.A. Simmons, J.M. Wotton*, M. Ferragamo*, and C.F. Moss[1]. Dept. of Psychology and Section of Neurobiology, Brown Univ., Providence, RI 02912 & [1]Dept. of Psychology, Harvard Univ., Cambridge, MA 02138

The big brown bat, *Eptesicus fuscus*, perceives images that incorporate both target range and shape onto a common psychological axis of range, defined in terms of echo delay (Simmons, J.A., *Cognition*, 33:155, 1989). Target range is represented by the timing of neural discharges encoding echo delay, while target shape (range separation of reflecting points) initially is represented from frequency-dependent stimulus amplitude (notches and peaks in echo spectra) but is transformed into equivalent time-domain information before being expressed in perceived images. The external ears of humans, cats, and FM bats similarly modify sounds entering the ear canal to encode the vertical position of sound sources by adding reverberation and spectral emphasis. As vertical angle changes, a prominent notch in the ear's spectral response moves to different frequencies (from 40 to 55 kHz in *Eptesicus*) and changes occur in the height of an adjacent spectral peak (around 60-65 kHz in *Eptesicus*). The bat incorporates external-ear effects into the images in perceives by transforming these spectral cues into equivalent time-domain information, just as it transforms spectral shape cues. The bat thus recovers external-ear reverberation-time as an estimate of time, not frequency. The resulting images express range, shape, and vertical position along a single, computed psychological axis of range, still defined in terms of echo delay. The neural representation of vertical position may be difficult to identify without consideration of the encoding of spectral notches and the transformation of spectral into temporal estimates. (Work supported by ONR grant N00014-89-J-3055 and NIH grant DC00511)

379.4

NEURAL PROCESSING OF PULSE REPETITION RATE AND PULSE DURATION IN THE BAT INFERIOR COLLICULUS. A.D. PINHEIRO, M. WU* AND P.H.-S. JEN Div. Bio. Sci. University of Missouri-Columbia,MO 65211

Encoding of stimulus repetition rate and duration by the inferior collicular neurons(IC) of the big brown bat, *Eptesicus fuscus* was studied by recording responses of each neuron to a wide range of repetition rates and durations at several stimulus intensities under free field stimulus conditions. A total of 277 IC neurons were recorded at depths between 147 and 2370 μm with latencies between 3.4 and 28.4 ms. They were tonotopically organized along the dorsoventral axis of the IC according to their best frequencies(BFs). While individual IC neurons varied their number of impulses but not their discharge pattern when stimulated with different repetition rates and durations, they generally discharged with a maximal number of impulses to a specific repetition rate (the best repetition rate) and duration (the best duration). According to the filtering properties for the stimulus repetition rate or duration, IC neurons can be classified as high-pass, low-pass, band-pass (or semi band-pass) and multipass neurons. A wide range of best repetition rates and best durations were identified for IC neurons but they were not correlated with either the BFs or recording depths. Futhermore, the best repetition rates and durations of tonotopically organized IC neurons isolated within an electrode penetration varied unpredictably. According to the correlation of their responses to stimulus duty cycle, IC neurons can be classified into two groups. Responses of one group correlated with the duty cycle thus enable the bat to avoid pulse-echo overlap during echolocation. Responses of the other group do not correlate with the duty cycle. These neurons likely enable the bat to encode the pulse emission rate and duration effectively during different phases of hunting. The effect of stimulus repetition rate and duration on the intensity rate function of IC neurons was examined. While the stimulus repetition rate might affect the dynamic range and overall profile of the intensity rate function curve, only little effect was observed with different stimulus durations.

379.5

SPECTRAL SELECTIVITY OF DELAY-SENSITIVE NEURONS IN THE AUDITORY CORTEX OF AN FM BAT. D. Wong and M. Maekawa, Anatomy Dept., Indiana Univ. School of Medicine, Indianapolis, IN 46202.

Spectro-temporal processing plays prominently in auditory perception. In the echolocating bat, *Myotis lucifugus*, delay-sensitive, cortical neurons process target-distance information in the time domain. The concurrent role of spectral processing in target perception was further examined neurophysiologically. Delay-sensitive neurons were isolated by stimulation with FM-FM sound pairs at frequency excursions mimicking this species' natural echolocation sounds (pulse) and echoes. The pulse and echo FMs were each arbitrarily divided into four spectral quarters and the quarter essential for delay-sensitivity was determined. Neurons typically required a spectral combination consisting of both the IV pulse-quarter and the III or IV echo-quarter. The essential echo component did not change when the rate of pulse-echo stimulation was increased. The preservation of this spectral combination for delay-sensitivity suggests that throughout echolocation FM bats employ specific parts of their pulse-echo spectra for perceiving target structure. (Supported by NIH grant DC00600).

379.6

EFFECT OF GONADAL STATE ON SEXUALLY DIMORPHIC BRAIN AREAS OF TWO SPECIES OF WHIPTAIL LIZARDS: A QUESTION REVISITED. J. Wade[1] and D. Crews[1,2] Departments of [1] Psychology and [2] Zoology, and the Institute of Reproductive Biology, University of Texas, Austin, TX 78712.

Cnemidophorus inornatus is a sexually reproducing species. The closely related *C. uniparens* is all-female and reproduces by parthenogenesis. However, *C. uniparens* consistently display reproductive behaviors common to both male and female *C. inornatus*. Both the anterior hypothalamus-preoptic area (AH-POA), a critical location of androgen action on mounting, and the ventromedial hypothalamus (VMH), where estrogen implants elicit receptivity, are sexually dimorphic in *C. inornatus*. The AH-POA is larger in males, while the VMH is larger in females. In *C. uniparens*, both brain regions are comparable in size to female *C. inornatus* (Crews et al., *Brain Behav. Evol.* In press). To investigate whether these brain areas change in volume with the reproductive state, the AH-POA and VMH were measured during the breeding season, during a simulation of winter hibernation and in gonadectomized male and female *C. inornatus* and *C. uniparens*. Neither brain area was significantly different in female *C. inornatus* or *C. uniparens* as a function of season or gonadal state. The AH-POA results in female *C. inornatus* were consistent with a previous study (Wade & Crews, *Soc. Neurosci. Abst.* 15: 1089). However, the VMH results did not replicate, perhaps due to procedural differences or different lengths of time the animals were in captivity. The magnitude of change in the female VMH volume was the same in both studies, but when corrected for brain size the second year, the difference was not statistically significant. In contrast, the AH-POA was smaller in hibernating and gonadectomized males than in reproductively active males (ANOVA: $F(2,22)=21.5$, $p<.01$). Conversely, the VMH was larger in hibernating and castrated than reproductively active males (ANOVA: $F(2,22)=7.2$, $p<.01$). In both cases the non-breeding males had brain areas that were not significantly different from those of intact females.

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379.7

CENTRAL MONOAMINES and BEHAVIOR IN THE LIZARD, *ANOLIS CAROLINENSIS* N. Greenberg[1], Cliff H. Summers[1], and P. H. Desan[2]. University of Tennessee, Knoxville, Graduate Program in Ethology[1]; Stanford University Medical Center[2].

To clarify monoamine function in stereotyped behavior, the selective neurotoxin MPTP was used to deplete dopamine (DA) and norepinephrine (NE) in a model species, the lizard, *Anolis carolinensis*.

Following the intraperitoneal injection of MPTP, animals manifested reduced spontaneous activity but normal or slighly facilitated aggression and reproductive behavior. Body color change, an index of altered autonomic sensitivity, suggested enhanced responsiveness.

Earlier work (Font *et al. 1987*) revealed MPTP-induced central neuropathies. Immunohistochemistry was employed to discriminate specific central catecholaminergic systems thereby affected. DA and NE immunoreactive perikarya were found in several sites in and near the periventricular hypothalamus, ventral tegmentum, substantia nigra, locus ceruleus, nucleus of the solitary tract, and raphe; while immunoreactive 5HT cells were found at one periventricular hypothalamic site and in the raphe nuclei.

Estimates of quantitative change in catecholamine levels obtained with high pressure liquid chromatography revealed that 100 hr after MPTP injection, DA and NE levels were 26% and 7% of control, respectively, while 5-HT was elevated to 200%.

379.8

EVENT RELATED POTENTIALS IN TECTUM AND CORTEX OF FREELY MOVING TURTLES TO VISUAL STIMULI. J.C. Prechtl and T.H. Bullock. Department of Neurosciences A-001., School of Medicine & Neurobiology Unit, Scripps Institution of Oceanography., UCSD, La Jolla, CA 92093

Pond turtles *(Chrysemys picta, Pseudemys scripta elegans)* were chronically implanted with 13-20 epipial electrodes on the midbrain and dorsal cortex. After recovery the turtles were placed in plastic chambers with 2 cm of water (20-23°C), and responses were recorded to single flashes and trains of flashes. Tectal visual evoked potentials (VEPs) with long interstimulus intervals (ISI=20 s) consist of a principal positive peak at ca. 95 ms (P95) and smaller waves. A change in the ISI from 20 s to 3 s, delivered for 1 min, attenuates the P95, peak-to-peak by 23±3% (SEM). The largest cortical VEPs were dominated by an N130 that showed a greater response attenuation (40±6%) at the 3 s ISI. Distinct from the tectum, the cortical VEP includes a late, broad (ca. 230-490 ms) negative wave especially sensitive to repetition rate; it declines by 68±6% with the shorter (3 s) ISI. Tectal VEPs to trains of flashes show 1:1 following up to ca. 20 Hz, without averaging. At 35 Hz in averages of 10 sweeps fusion is complete, whereas, from the cortex fusion occurs at less than 25 Hz.

As in the fish tectum (Bullock et al. 1989, *Soc. Neurosci. Abstr.*, 15:1137) event related potentials are evoked in the turtle tectum and cortex by the omission of a flash after a conditioning series. The latency of this omitted stimulus potential (OSP) after the due-time of the first missing flash tends toward a constant, ca. 50 ms, as though the brain had learned an expectation. Jitter in the ISI however, does not reduce the OSP greatly. The form of the OSP is not the same in the tectum and cortex. Its amplitude increases with conditioning flash rate, which must be minimally 3 Hz for tectal OSPs, <2 Hz for cortical OSPs. On some electrodes, after certain conditioning frequencies, the OSP included conspicuous oscillations between 16 and 25 Hz. The findings suggest that the OSP reflects a release from an accumulated inhibition, hence endogenous processes although minimal cognition.

379.9

INVOLVEMENT OF MECHANOSENSORY LATERAL LINE SYSTEM IN FEEDING BEHAVIOR OF THE AXOLOTL. H.-A.Takeuchi[1], H.Namba[1]* and T.Nagai[2]. [1]Dept. Biol., Fac. Sci., Shizuoka Univ., Shizuoka 422, Japan, [2]Dept. Physiol., Teikyo Univ. Sch. Med., Tokyo 173, Japan.

Axolotls (Ambystoma mexicanum) exhibit a series of feeding behavior to the prey objects moving in the water. The feeding behavior is elicited by the artificial moving object. To understand the neural mechanisms of thus elicited feeding behavior, we identified the effective parameters of the vibrational stimuli applied to the water. Feeding behaviors were compared between normal and genetically blind (eyeless) axolotls. Then, we studied the effects of blocking the lateral line system on the feeding behavior. We used two methods to block the lateral line system: 1) the exposure to cobalt solution [Co treatment] and 2) the transection of ramus ophthalmicus superficialis (r.o.s) and ramus buccalis (r.buc.) of anterior lateral line nerve [ALL transection]. We also examined the central projection of r.o.s and r.buc. by using the transganglionic transport of cobaltic lysine.

The feeding behavior was evoked by the water movement produced by the vibrating sphere (frequency: 2-35Hz; amplitude: 1-4mm). The vibrational stimuli were effective in normal as well as blind axolotls, suggesting that sensory system other than vision was involved in the feeding behavior. In the blocking experiment of lateral line system, the feeding responses were significantly reduced in the animals exposed to 0.05-1.0mM Co^{2+} solution. Experiment of ALL transection showed that the transection of r.o.s. and r.buc. significantly suppressed the feeding behavior. The present results suggest that the mechanosensory signals mediated by anterior lateral line nerve (r.buc. and r.o.s.) are involved in the release of feeding behavior in the axolotl.

379.10

ROLE OF PHASIC NEURONS IN THE PROCESSING OF TEMPORAL INFORMATION IN THE FROG AUDITORY SYSTEM. C.J. Condon[1], S.-H. Chang, D.M. Gooler, J.C. Hall, W.Y. Lin*, K.R. White, and A.S. Feng. [1]Neuroscience Program, and Dept. of Physiology and Biophysics, Univ. of Illinois, Urbana, IL 61801.

While phasic neurons are considered to be essential for encoding the time of occurrence of a sensory event, other specific functions these neurons serve in acoustic information processing have not been fully elucidated. Given that various temporal parameters [e.g. rise-fall time, duration, and rate of amplitude modulation (AM)] of frog calls serve as essential cues for call discrimination, one might predict that phasic neurons are likely to be involved in the coding of temporal patterns of complex sounds. With this as a working hypothesis, we have systematically recorded single unit activity at various levels in the auditory system of the leopard frog (*Rana p. pipiens*) in order to characterize the role of phasic neurons (relative to tonic neurons) in the encoding of such behaviorally significant temporal sound parameters.

With respect to the processing of signal rise-fall time and duration, phasic neurons in the auditory brainstem nuclei (dorsal medullary, superior olivary, and torus semicircularis) develop temporal selectivities typically not manifested by tonic neurons. For example, many neurons that were found to be "tuned" to a narrow range of AM rates (i.e. BAND-PASS response functions) were phasic responders in the various auditory stations examined. Thus, in addition to coding for the arrival time of an acoustic stimulus, phasic neurons also act as "temporal filters" with respect to rise-fall time, signal duration, and the rate of AM. This suggests that the temporal selectivity of phasic neurons for various temporal parameters may contribute to the recognition of complex sounds in the acoustic communicative behavior of frogs and perhaps other species. [Research supported by NSF grant BNS 88-09490]

379.11

THE INFLUENCE OF SOUND DIRECTION ON PROCESSING OF COMPLEX SOUNDS BY MIDBRAIN AUDITORY NEURONS. D.M. Gooler, C.J. Condon[1], and A.S. Feng. Dept. of Physiology and Biophysics, and [1]Neuroscience Program, University of Illinois, Urbana, IL 61801.

Head and body orientations are important for acoustic communication in complex environments. Whether animals change orientations to improve sound localization, and/or to aid the detection of complex sound features is not clear. In the complex acoustic environment of a breeding pond, frogs can discriminate between different signals as well as perceive the locations of multiple sources. We are interested in the mechanisms by which the frog's nervous system forms a coherent image of acoustic space. This study focuses on the influence of sound direction on the neural processes underlying sound pattern recognition, especially those of behaviorally meaningful sounds.

We have recorded from single neurons in the auditory midbrain, the torus semicircularis (TS), of leopard frogs to investigate the influence of sound direction on frequency tuning characteristics (FTCs) and responses to amplitude modulated sounds. Free field acoustic stimuli were presented to the frog from equidistant azimuthal locations. The process of encoding sound pattern and direction by neurons in the TS appears to be complex. In many cases a change in sound direction elicited pronounced changes in the FTCs and modulation transfer functions (MTFs) of individual TS neurons. Most notable changes were shifts in the unit's characteristic frequency (CF), threshold at CF, and shape of the FTC and the MTF. The relationship between sound incident angle and specific changes in the features of the FTCs and MTFs varied from cell to cell. In contrast, some neurons showed no change in CF or MTF with a change in sound direction.

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379.12

COMPARISON OF TIME-LOCKED RESPONSES OF FROG'S CENTRAL AUDITORY NEURONS TO SINUSOIDAL-AMPLITUDE-MODULATED SIGNALS WITH PHASE-LOCKED RESPONSES TO SINUSOIDAL CARRIERS. W.-Y. Lin*, K.R. White, and A.S. Feng. Department of Physiology and Biophysics, University of Illinois, Urbana, IL 61801.

Single unit recordings were made from the frog's dorsal medullary nucleus (DMN), a homolog of cochlear nucleus, to determine whether or not the phase-locked response capacity of a central auditory neuron to sinusoidal stimuli can be used to predict the time-locked response capacity to sinusoidal-amplitude-modulated (SAM) signals, and vice versa. For each cell, we first determined the best excitatory frequency (BF) and the frequency tuning curve (FTC) of the unit. We then quantitatively analyzed the unit's responses to: (1) tone bursts at a number of frequencies within the unit's FTC at 10 dB above the respective thresholds of excitation, and (2) SAM stimuli at various modulating frequencies (ranging from 5Hz to half of the BF or 1000 Hz, whichever was less) using the unit's BF as the carrier at 10 dB above the excitatory threshold at BF. The synchronization coefficient (SC) was calculated from the period histogram derived from each of these responses using the formula given by Goldberg and Brown (1963). The SCs from time-locked and phase-locked responses at comparable frequencies (modulating vs carrier frequency) were compared.

We found that for mid- to high- frequency neurons (BF>700 Hz), SCs for SAM stimuli were typically lower than SCs derived from phase locked responses to sinusoidal carriers of comparable frequencies. For low-frequency sensitive neurons (BF=100-650 Hz), the SCs derived for the two types of stimuli at comparable frequencies were similar.

(Research supported by a NSF grant 88-09490)

379.13

WITHIN AND BETWEEN POPULATION VARIATION IN THE ACOUSTIC COMMUNICATION SYSTEM OF CRICKET FROGS. A. C. Keddy-Hector,* W. Wilczynski, and M. J. Ryan.* Depts. of Psychology and Zoology, Univ. of Texas, Austin, TX, 78712.

We compared variation in basilar papilla (BP) tuning and call dominant frequency in populations of Acris crepitans from Wimberley (WB) and Stengel Ranch (SR), Texas. The best excitatory frequencies of BP afferents were obtained in 28 females and 16 males, calls recorded from 107 males, and snout-vent lengths measured in all individuals. In both populations, females were tuned lower than males and lower than the dominant frequency of male calls. An inverse relationship existed between body size and BP tuning in both sexes (WB male r= -0.38, female r= -0.57; SR male r= -0.62, female r= -0.64). However, analysis of covariance showed that body size differences do not account for the sex difference in BP tuning (WB F=9.32, df=1,20; SR F=4.5, df=1,15). The analysis also showed that tuning and calls differ between populations independent of body size. The slopes of female tuning or male calls vs. body size did not differ in the two populations, but the y-intercepts did (F=728, df=1,12; F=4.92, df=1,104). Slopes of male tuning vs. body size differed between populations (F=7.35, df=1,13). These results suggest that in cricket frogs interpopulational differences in vocal and auditory traits result from concomitant shifts in the intrapopulational allometry of these traits with body size. (Supported by NIMH 1T32 MH18837 and NSF BNS 8606289.)

379.14

SEXUALLY DIMORPHIC LARYNGEAL MORPHOLOGY IN CRICKET FROGS (ACRIS CREPITANS). B. E. McClelland, W. Wilczynski and M. J. Ryan*. Depts. of Psychology and Zoology, University of Texas, Austin, TX 78712.

In every frog species studied so far, male larynges are significantly larger than females', but differences exist among the species in the relative development of the female larynx. The degree of sexual dimorphism in the laryngeal morphology of a species may be related to the presence or absence of vocalizations in the female's behavioral repertoire. Because female cricket frogs (Acris crepitans) are completely silent, we predict extensive quantitative and/or qualitative laryngeal differences between the sexes.

In this study we used morphometric techniques to explore the differences between the anatomy of male and female larynges. Although the body size [snout-vent length: male (n=13) =2.30 mm, female (n=8) =2.24 mm] and head widths of the males (.79 mm) and females (.78 mm) were not significantly different, the volume of each male (M) laryngeal component was much larger than the corresponding feature of the females' (F) larynx (arytenoid cartilage: M=.68, F=.046; vocal cord: M=.048, F=.004; constrictor muscle: M=.64, F=.064; dilator muscle: M=.182, F=.023; all volumes mm^3). Female vocal cords were rudimentary and did not stretch across the laryngeal lumen which may have rendered them non-functional. Within each sex, the volumes of all the laryngeal features are positively correlated with body size. However, the slopes describing the male relationships are, in all cases, much steeper than the females'. Additionally, within each sex, the size relationships among the laryngeal components appear to be the same. In summary, although male larynges are considerably larger, the anatomical components of male and female larynges maintain the same allometric relationship within the sex. Not only the small size, but also the lack of vocal cord development may prevent females from vocalizing. (Research supported by NSF BNS 8606289.)

379.15

ACOUSTICALLY SUBOPTIMAL MALE SPACING IN HYLA CINEREA CHORUSES **J. H. Fox, W. Wilczynski, and W. M. Moss*. Dept. of Psychology, University of Texas, Austin, TX 78712**

Previous computer models of frog chorus acoustics have revealed that males can maximize per-capita mating call active space by utilizing "optimal" neighbor spacing (Fox and Wilczynski, 1986. SN Abst. 12:314). Here, we examine whether local green treefrog (Hyla cinerea) males utilize this strategy. For this species, optimal **interindividual distance** (**IID**) depends on **threshold distance** (**TD** -- the distance at which a female can just detect a male's call), **call duty cycle** (**DC** -- the proportion of time the male produces sound), and **chorus population**. TD was determined by filtering calibrated call FFTs with female audiograms, derived from multiunit auditory midbrain (torus semicircularis) responses to sinusoidal acoustic stimuli. DC was measured by playing tape recordings of long calling bouts into an electronic timing apparatus. We find that TD = 377 ± 181 m, DC= .128 ± .033, and chorus population is typically ca. 10 - 20. Therefore, computer models predict an optimal IID of 310 m. Actual IID, 8.21 ± 7.28 m, is much lower than optimal. Such a large optimal IID must be impractical for H. cinerea, because the resulting chorus would be too large for the breeding area and would be difficult for females to traverse; therefore, "optimal spacing" would have little biological relevance. It now seems likely that active space maximization through use of optimal IID may be feasible only for species with lesser TD values, such as Acris crepitans (Fox, 1988. SN Abst. 14:88). (Supported by NSF BNS 8606289.)

379.16

REPRODUCTION AND FEEDING OF THE FEMALE FROG, RANA PIPIENS AFTER ADMINISTRATION OF YOHIMBINE, CLONIDINE, AND NEUROPEPTIDE Y. C. Diakow, M.C. Lanzillotta* and J.T. Clark, Biol. Dept., Adelphi U., Garden City, NY 11530 and Physiol. Dept. Meharry Med. Col., Nashville, TN 37208.

This study provides evidence that both α_2 receptors and neuropeptide Y(NPY) play roles in enhancing reproductive behavior of the female frog, and that α_2 receptors play a role in reducing the tendency to feed.

When females become receptive to males there is a decline in the tendency to emit a vocalization, the release call, in response to tactile stimulation. In this experiment, the number of release calls in response to manual stimulation declined after administration of 0.08 µg/g yohimbine, or 0.001769 nmoles/g NPY, but not after injection of 0.08 µg/g clonidine. Injections were administered through cannulae surgically implanted in the 3rd ventricle. Control and drug injections were counterbalanced one day apart. Each female received only one drug and there were eleven, 30 sec. release call tests between 5 min. and 3 hr. after injection.

Between tests for the release call, females were placed in a tank with crickets. The number of crickets eaten was not affected by drug treatment, but the latency to eat the first cricket was highest after yohimbine injection.

380.1

TESTOSTERONE IMPLANTS RESTORE STEROID-SENSITIVE VASOPRESSIN INNERVATION IN CASTRATED RATS. G.J. De Vries, and R.M. Buijs*, Prog. in Neurosci. and Behav., Univ. of Mass., Amherst, and Netherlands Inst. for Brain Res., Amsterdam.

Vasopressin-immunoreactive (AVP-IR) projections of the bed nucleus of the stria terminalis (BST) and the medial amygdaloid nucleus (MA) are steroid-sensitive. Gonadectomy wipes out AVP staining while subsequent testosterone treatment restores it. We studied whether unilateral implantation of testosterone in and around the BST and MA could restore this staining in long-term castrated rats.

Implants in the BST of long-term castrated rats restored most of the innervation at the side of the implant, but there were differences depending on where the implants were placed. For example, implants in medial regions of the BST restored most of the original fiber staining in the lateral septum but not the staining in the lateral habenular nucleus. Implants in lateral regions of the BST restored some fiber staining in the lateral habenular nucleus. Most fiber staining in the lateral habenular nucleus was restored, however, by implants in the MA. Since implanting testosterone between the BST and MA did not restore any fiber staining, the steroids acted probably locally in both nuclei. The differences in the effects of the implants might be directly related to differences in projections of subgroups of AVP cells in the BST and MA. Steroid implants may restore AVP fiber staining only in areas that receive direct AVP projections from the site of the implant. Alternatively, steroid implants might have stimulated other steroid-sensitive neuronal systems that, in turn, might have activated steroid-sensitive AVP neurons transsynaptically. This would explain why implants in the MA restored much of the AVP innervation of the lateral septum, even though the MA projections to the lateral septum are much less dense than those of the BST.

380.2

SPECIES AND SEX DIFFERENCES IN VASOPRESSIN PATHWAYS OF VOLES THAT SHOW DIFFERENT PATTERNS OF PARENTAL CARE. M. Bamshad, G.J. De Vries, M.A. Novak*, Prog. of Neurosci. and Behav. and Dept. of Psychol., Univ. of Mass., Amherst, MA 01003.

Prairie voles (*Microtus ochrogaster)* spend longer time in close contact with their young than meadow voles (*Microtus pennsylvanicus*). In addition, meadow vole males do not show any parental care towards their young, whereas prairie vole males do. In rats, body temperature of the mother appears to determine how long she nurses the young during each nesting bout (Leon M, *Phys. Behav.* 1978 21:793). Since vasopressin-immunoreactive (AVP-IR) projections of the bed nucleus of the stria terminalis (BST) and medial amygdaloid nucleus (MA) have been linked to body temperature regulation (Pittman Q, *Brain Res. Bull.* 1988 20:887) we studied whether there were differences in these projections that could be related to the aforementioned sex and species differences.

Brains of sexually inexperienced voles and voles that were six days postpartum were stained immunocytochemically for vasopressin. In both species, AVP-IR projections of the BST and MA were much denser in sexually inexperienced males than in females. This pattern was changed in parental animals. In females of both species, AVP-IR projections of the BST and MA were just as dense as in sexually inexperienced males. In meadow vole males, there were no differences between parental and inexperienced males, whereas in prairie vole males BST and MA projections showed less staining in parental animals. These findings suggest that, in females, BST and MA projections are involved in changes that take place when animals become parental. The sex difference in the direction of changes in parental prairie voles that become parental suggest that vasopressin might serve different functions related to parental behavior in both sexes.

380.3

GABA IN THE MIDBRAIN CENTRAL GRAY FACILITATES LORDOSIS IN THE FEMALE RAT. M.M. McCarthy, D.W. Pfaff and S. Schwartz-Giblin, Rockefeller University, New York, NY 10021

The inhibitory neurotransmitter GABA has been implicated in the control of female reproductive behavior. Microinfusion of the GABA-A agonist, muscimol, into POA inhibits lordosis whereas muscimol into medial hypothalamus facilitates lordosis (McCarthy et al, Brain Res. 507: 1990).

Using a 2-chamber apparatus in which a sexually active male is restricted to one chamber, several parameters of female sexual behavior were measured during 10 min tests. After pre-testing, steroid-primed females were infused with GABAergic drugs via bilateral cannulae (0.25µl/side) in the midbrain central gray (MCG). In estrogen (E) + progesterone (P)-primed females with high lordosis quotients during pre-tests, the GABA-A antagonist, bicuculline, significantly reduced lordosis and proceptive behaviors by 5 min at a dose of 30ng but not 10ng ($p<.05$; Wilcoxon Test). By one hour post-infusion, lordosis quotients were equal to pre-test levels. Infusion of muscimol (50ng) did not affect behavior in E + P-primed females. Conversely, with low lordosis quotients and high avoidance index (# rejections / # mounts) in E-primed females, muscimol (50ng) significantly increased lordosis quotient while decreasing avoidance index as compared to saline-infused controls at 5 and 60 min post-infusion ($p<.05$; Mann-Whitney U). Locomotion and time spent in the male's chamber did not change after treatment with either drug.

Infusion of the retrograde tracer Fluorogold (0.25µl) through each cannula after behavioral testing showed strong descending projections to MCG from the anterior hypothalamus, zona incerta, ventromedial nucleus of the hypothalamus and amygdala (central and medial nuclei). Ascending projections from lumbar cord included cells in laminae I, V, X and lateral spinal nucleus. Therefore, in addition to being facilitatory in the medial hypothalamus, GABA also facilitates lordosis at MCG sites which receive behaviorally relevant afferent inputs from forebrain as well as spinal cord.

380.4

GABAERGIC CONTROL OF SEXUAL RECEPTIVITY IN THE FEMALE RAT. D.B. Masters, J.M. Fiber and M.M. McCarthy, Institute of Animal Behavior, Rutgers University, Newark, N.J. 07102 U.S.A.

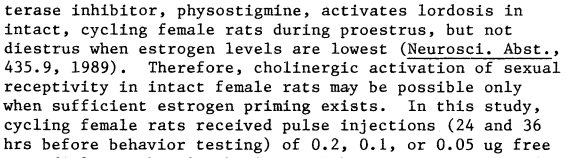
Recently, we reported that GABA agonist infusion into medial hypothalamus (mHYP) and preoptic area (POA) has facilitory and inhibitory actions on lordosis behavior, respectively (McCarthy et al, Brain Res. 507, 1990). These data suggest that GABA differentially mediates receptive behavior. Therefore, in this study we hypothesized that endogenous GABA levels will be greater in mHYP than in POA in receptive (Rec) and opposite in non-receptive (NRec) females along with concomitant changes in GABA receptor binding.

Intact and ovariectomized (OVX) rats were tested for receptivity 24 and 1 hr before their mHYP, POA & cortex (CTX) were dissected & frozen (liq N_2). Sections were homogenized in 1 ml 4% perchlorate & 50 µM norvaline (int std.), centrifuged, and the supernatant was measured for GABA (blinded procedure) by high pressure liquid chromatography (HPLC).

The results (fig. 1), normalized by [CTX-GABA], show a significant* difference between mHYP and POA [GABA]/mg tissue in Rec & NRec females. The [CTX-GABA] was unchanged ($p > 0.83$). The mHYP/POA GABA ratio was significantly* greater in Rec vs NRec & OVX rats, 1.34 ± 0.07, 0.90 ± 0.08, & 0.88 ± 0.09, respectively (avg ± sem). In a parallel experiment ^{3}H-muscimol binding (Bmax) to the high affinity receptor site was significantly* lower in POA of Rec vs NRec & OVX rats. Receptor affinity in mHYP of Rec rats was significantly* less (3 fold greater K_D) than NRec & OVX groups.

In conclusion, the data support our hypothesis that GABA plays a dual role in control of female mating behavior. Increased GABAergic activity in mHYP with a concurrent reduction in POA activity promotes receptivity, whereas the reverse inhibits this behavior. It is possible that tonic GABA levels and receptor dynamics in mHYP and POA are modulated by systemic steroids associated with the estrus cycle, regulating the natural onset and termination of receptive behavior in female rats. *ANOVA ($p < 0.05$) was used.

Fig 1.



380.5

STEROID MODULATION OF CHOLINE ACETYLTRANSFERASE ACTIVITY IN THE ZEBRA FINCH BRAIN. A. Gardner* and C.F. Harding, Biopsychology Program, Hunter College, CUNY, New York, NY 10021.

Acetylcholine appears to be present throughout the songbird's vocal control system. Vocal control nuclei (VCN) concentrate steroids, altering neural functioning and activating behavior. Cholinergic neurons have been implicated in the control of steroid-mediated behaviors in other species, and cholinergic enzyme activity in the finch syrinx is hormone dependent. This study examined the effects of androstenedione (AE), the steroid hormone we find best restores hormone-dependent behavior in castrated finches, on cholinergic enzyme activity in the finch brain.

Castrated males received silastic implants of cholesterol or AE and were then paired with females in individual cages for one week prior to sacrifice. Choline acetyltransferase (CAT) activity was measured in 10 steroid-sensitive nuclei (VCN: RA, HVc, DM, NIf, MAN, Area X; auditory area: Field L; hypothalamic nuclei: POA, PVM, IN). CAT activity was measured by radioenzymatic assay and expressed in terms of specific activity, nM substrate/mg protein/hour.

As expected, steroid treatment affected CAT activity in several of the VCN studied. Interestingly, unlike the effects of steroid hormones on catecholamines, which increased neurotransmitter levels and turnover in some areas but decreased them in others, AE always increased CAT activity in those brain areas in which it exerted an effect.

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380.6

ESTROGEN-DEPENDENT CHOLINERGIC REGULATION OF LORDOSIS IN INTACT CYCLING FEMALE RATS. C.S. Menard and C.P. Dohanich. Dept. of Psychology, Tulane Univ., New Orleans, LA 70118.

Intracerebral administration of the acetylcholinesterase inhibitor, physostigmine, activates lordosis in intact, cycling female rats during proestrus, but not diestrus when estrogen levels are lowest (Neurosci. Abst., 435.9, 1989). Therefore, cholinergic activation of sexual receptivity in intact female rats may be possible only when sufficient estrogen priming exists. In this study, cycling female rats received pulse injections (24 and 36 hrs before behavior testing) of 0.2, 0.1, or 0.05 ug free estradiol or ethanol vehicle at either Diestrus I or Mid-diestrus. On the following day, females that were not sexually receptive (LQ < 50%) received intraventricular infusions of either saline or physostigmine (10 ug bilaterally) and then were tested for lordosis. Lordosis was increased 15 min ($p < .0001$) and 1 hr ($p < .0063$) after physostigmine infusion on both days. The level of facilitation at 15 min ($p < .0001$) and 1 hr ($p < .0212$) increased with the levels of estradiol priming. No facilitation was evident with ethanol priming on either day. These results indicate that in intact female rats, cholinergic systems that regulate sexual behavior are dependent upon sufficient estrogen priming. Furthermore, estrogen levels above normal diestrus levels appear to be necessary for cholinergic regulation to occur.

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380.7

SEXUAL DIFFERENTIATION AND MONOAMINERGIC SYSTEMS: STEROID INDUCED BEHAVIORAL AND NEUROCHEMICAL CHANGES. M.A. Abdelnabi*, A. Powell+, C. Rexroad+ and M.A. Ottinger*. * Dept of Poultry Science, Univ. of Maryland, College Park, MD 20742 and +Reproduction Lab, USDA-ARS, Beltsville, MD 20705

Monoaminergic systems and sexual differentiation in Japanese quail were studied by 1) assay of hypothalamic monoamine content and turnover during development and 2) study of long term effects of exogenous steroids on neurochemistry and behavior. In Study 1, female embryonic and perinatal quail had higher levels of norepinephrine (NE) (382.8 ng/g) than males (160.8 ng/g) at day 10 prehatch, while males had higher levels of DA (16.1 and 40.9 ng/g) than female at Days 12 and 16 respectively. 5-HT was higher (25.6 ng/g) in females at Day 14 prehatch, while males showed high levels (1107 and 1416 ng/g) at Day 5 and 7, posthatch. Turnover rates were significantly higher for both NE and DA (51.1 and 54 ng/g/hr) at Day 7 posthatch, respectively and differed with sex. In Study 2, avian embryos, treated on one of 9 ages with estradiol, testosterone or oil showed long term effects on male behavior. Preoptic area monoamine content showed that females had higher levels of NE and 5HT than males. These results demonstrate permanent alteration of the monoamine system with early exposure to steroid hormones. These data indicate that steroid induced behavioral responses in the adult may rely on steroid induced alterations in neurotransmitter systems during sexual differentiation.

380.8

NORADRENERGIC REGULATION OF SONG AND COURTSHIP IN MALE ZEBRA FINCHES. S. R. Barclay, S. A. Waterman and C. F. Harding. Biopsychology Program, Hunter College, CUNY, New York, N.Y. 10021.

Previous studies have suggested that catecholamines (CA) may be involved in controlling courtship behaviors in male zebra finches. Hormone treatments which increased courtship behaviors in castrated males significantly altered CA levels and turnover in both hypothalamic and vocal control nuclei. Similar changes in CA function were seen during the initial phases of the reproductive cycle. While hormone treatment affected both norepinephrine (NE) and dopamine (DA) function, the changes in NE were much more striking. We therefore decided to manipulate NE levels and examine the effects on courtship behavior.

Intact male finches were pretreated with zimelidine and then injected with N-(2-chloroethyl)-N-ethyl-2-bromo-benzylamine (DSP-4, 50μg/g) or saline ip. Birds were observed for 15 minutes in pre- and post-drug pair tests. Vocal control and hypothalamic nuclei were assayed for NE, DA and serotonin (5-HT) using HPLC-EC. DSP-4 birds showed significant post-drug decrements in total courtship behavior. This treatment increased the latency to sing and decreased the total number of song bouts, while the motor patterning of songs appeared unaffected. As expected, DSP-4 significantly depleted telencephalic (i.e., vocal control nuclei) NE while hypothalamic NE was not affected. Zimelidine pretreatment prevented significant changes in DA and 5-HT levels. These results suggest that the noradrenergic system is important in modulating courtship vocalizations in the zebra finch.

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380.9

EFFERENT PROJECTIONS FROM THE VENTROLATERAL HYPOTHALAMUS IN FEMALE GUINEA PIGS. K.H. Nielsen and J.D. Blaustein, Neuroscience and Behavior Program and Psychology Department, University of Massachusetts, Amherst, MA 01003.

Implants of estradiol in the ventrolateral hypothalamus (VLH) are sufficient to prime ovariectomized guinea pigs to show progesterone-facilitated lordosis. To determine the efferent projections from this area, *Phaseolus vulgaris* Leucoagglutinin (Pha-L) was applied iontophoretically into the rostral VLH of ovariectomized guinea pigs. After a survival period of two weeks, the Pha-L was visualized immunocytochemically. The majority of Pha-L labelling was observed ipsilateral to the injection site, but occasional axons were labelled in the same contralateral structures. The following areas received the heaviest projections: bed nucleus of the stria terminalis, medial preoptic area, anterior hypothalamus, tuber cinereum, ventromedial nucleus, dorsomedial and dorsolateral hypothalamus, posterior hypothalamus, dorsal longitudinal fasciculus, midbrain central gray, and the recess inferior colliculus. The latter three areas also received the heaviest contralateral projections. Areas with fewer labelled terminals and fibers included the diagonal band of Broca, lateral septum, medial and cortical amygdala, lateral preoptic area, paraventricular nucleus, arcuate nucleus, premammillary nucleus, dorsal tegmental area, and pontine central gray. Fibers with few or no terminals were observed in the median forebrain bundle, stria terminalis, and medial lemniscus. Many of these projections are similar to the efferents from the ventrolateral-ventromedial nucleus in rats, the area apparently analogous to the VLH in guinea pigs.

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380.10

VENTROLATERAL HYPOTHALAMUS PROJECTIONS TO ESTROGEN RECEPTOR-IMMUNOREACTIVE SITES IN THE FEMALE GUINEA PIG BRAIN. J.C. Turcotte, K.H. Nielsen and J.D. Blaustein, Neuroscience and Behavior Program and Psychology Dept., University of Massachusetts, Amherst, MA 01003

The ventrolateral hypothalamus (VLH), an area in which implantation of estradiol is sufficient to prime guinea pigs to display progesterone-facilitated sexual behavior, contains a large number of estrogen receptor-immunoreactive (ER-IR) cells. In rats, projections from the analogous ventrolateral-ventromedial nucleus, have been traced to a variety of brain sites which contain estradiol-concentrating cells. These projections may be part of a neural network which may be involved in the regulation of sexual behavior. In order to determine if efferents from the VLH project to ER-IR neurons, we iontophoretically applied the anterograde tracer, *Phaseolus vulgaris* Leucoagglutinin (Pha-L) and visualized projections from this area with a double label immunocytochemical procedure. Pha-L injection sites were small and localized in sites containing a high concentration of ER-IR cells. VLH efferents were observed in many sites throughout the brain (Nielsen and Blaustein, 1990 Neurosci. abst.), some of which also contain ER-IR neurons. The ER-IR containing areas which receive VLH projections include the medial preoptic area, the periventricular area and the midbrain central gray. Within these areas, some projections were found closely associated with ER-IR neurons suggestive of synaptic contacts. This finding supports the concept of a neural network of estradiol-sensitive neurons.

(Supported by NIH NS 19327 and NS 00970)

380.11

EFFECTS OF PVN LESIONS ON THE RESPONSIVENESS OF OVARIECTOMIZED RATS TO ESTRADIOL P.C. Butera, D.M. Willard* & S.A. Raymond*. Dept. of Psychology Niagara University, NY 14109

This experiment tested the effects of estradiol on ingestive behaviors and body weight in female rats with bilateral lesions of the hypothalamic paraventricular nucleus (PVN). Thirty-two adult females received either bilateral or sham PVN lesions. All subjects were ovariectomized two weeks after the lesion. Two weeks following ovariectomy, half the animals were injected with 2 ug of estradiol benzoate (EB) for 3 days and half were injected with the oil vehicle. After 10 days of recovery the hormone treatments were reversed. Histological analysis indicated that 9 females had bilateral PVN lesions and 4 had bilateral lesions of the dorsomedial hypothalamus (DMH). Eleven animals received sham lesions. There were no significant effects of oil injections on food intake, water intake, or body weight. EB significantly lowered water intake and body weight in all groups, however, food intake was suppressed in the DMH and Sham but not PVN-lesioned females. These findings indicate that the effects of estradiol on feeding behavior are attenuated by PVN lesions. In addition, the results support the hypothesis that the effects of estradiol on food intake are mediated by its actions in the PVN.

(Supported by NIH NS26020)

380.12

HYPOTHALAMIC LESIONS FACILITATE THE DISPLAY OF SEXUAL BEHAVIOR IN THE FEMALE GOLDEN HAMSTER INDEPENDENTLY OF PHOTOPERIOD. A.S. Elliott* and A.A. Nunez, Dept. of Psych., Neuroscience Program, Michigan State University, East Lansing, MI 48824.

In order to further elucidate the role of the suprachiasmatic nucleus (SCN) in photoperiodic control of socio-sexual behaviors, electrolytic lesions aimed at the SCN were made in female golden hamsters (Mesocricetus auratus) and the animals were then placed in either a long or short photoperiod. After ovariectomy, the estrogen-primed animals with lesions showed lordosis more frequently than those without lesions, regardless of photoperiod. No differences were detected when the estrogen treatment was supplemented with progesterone. Animals with lesions also attacked less frequently than controls, again regardless of photoperiod. Histology revealed that the actual placement of the lesions ranged from the preoptic area through the caudal aspects of the SCN. Of the lesion group, those with damage in the anterior hypothalamus (AH), including the SCN, showed lordosis more frequently than those with damage solely in the preoptic area (POA). Conversely, those with AH lesions showed less aggression than those with POA lesions. These data support the findings that the POA, AH and SCN may play a role in mediating the inhibitory action of the septal-POA on lordosis in female hamsters (*Brain Res.* 1980, **181**:267; *Physiol. Behav.* 1982, **29**:1131).

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380.13

PREOPTIC AREA (POA) KNIFE CUTS AND KNIFE CUTS POSTERIOR TO THE VENTRAL TEGMENTAL AREA (VTA) DISRUPT MATERNAL BEHAVIOR IN RATS. M. Numan, M.J. Numan* and C.E. Lupini*. Dept. of Psychology, Boston College, Chestnut Hill, MA 02167.

The present series of experiments explored the possibility that POA projections through the VTA to lower brainstem regions are critical for maternal behavior in postpartum lactating rats. The first experiment found that bilateral coronal knife cuts posterior to the VTA depressed all aspects of maternal behavior: Retrieving, nest building and nursing behavior. In a second experiment, it was found that a parasagittal unilateral knife cut lateral to the medial POA paired with a contralateral coronal knife cut posterior to the VTA also disrupted maternal behavior, suggesting that POA projections through the VTA to lower regions may be important. In a final anatomical study, animals received knife cuts posterior to the VTA or served as controls. Horseradish peroxidase (HRP) was immediately injected into the knife cut region. Animals with the cuts had more HRP labeled cells in the POA than animals without such cuts, suggesting that the cuts did indeed sever descending POA efferents. However, in the animals with cuts many other brain regions were also labeled with HRP. One functionally relevant area was the sensory trigeminal nucleus. This system may be particularly important for retrieving behavior which is influenced by orosensory input.

Supported by a Whitehall Foundation grant.

380.14

TECTORETICULAR NEURONAL ACTIVITY ASSOCIATED WITH BEHAVIOR AND PROGESTERONE ACTION DURING THE INDUCTION OF LORDOSIS RESPONDING IN HAMSTERS. J. D. Rose. Depts. of Psychology and Zoology-Physiology, Univ. Wyoming, Laramie, WY 82071.

Superior colliculus neurons with antidromically-identified projections to the medial medulla were recorded in estrogen-primed golden hamsters during the induction of lordosis by systemic progesterone (P) injection. A crystalline progesterone-horseradish peroxidase conjugate (P-HRP) was applied to the superior colliculus with a removable cannula, near the location of the recording electrode array. P-HRP was used to distinguish non-genomic from genomically-mediated P effects on tectoreticular neurons. Rapid effects of P-HRP and more slowly-developing effects of systemic P were seen, including changes in firing levels, antidromic excitability and antidromic spike configuration. Tectoreticular neurons that fired actively during head movements ceased firing and displayed antidromic spike blockade during lordosis. Other tectoreticular neurons showed changes in antidromic spike configuation during movements, that differed from configuations present during episodes of lordosis. These results identify specific patterns of tectoreticular neuronal activity in relation lordosis and active movements. The activity and excitability of tectoreticular neurons during induction of lordosis appears to be regulated by a combination of membrane and genomic P effects. Supported by NIH Grant NS13748.

380.15

GUINEA PIG PERINEAL MOTONEURONS ARE SEXUALLY DIMORPHIC IN SIZE BUT NOT NUMBER. Louise M. Freeman & S. Marc Breedlove, Psychology Dept., U.C. Berkeley, Berkeley CA 94720.

Motoneurons (MNs) innervating the bulbocavernosus (BC), levator ani (LA) and ischiocavernosus (IC) muscles of male guinea pigs are located in the ventral horn of the S1-L6 spinal cord. Caudally these neurons form a single cluster, but more rostrally they separate into two distinct motoneuronal columns, with BC and LA MNs in the medial cluster and IC MNs in the lateral group. Female guinea pigs have a muscle that appears homologous to but smaller than the LA of males; MNs of this muscle, like those of the male LA, are seen predominantly in the medial column. There is no obvious IC in adult female guinea pigs.

To determine whether these MNs were sexually dimorphic in number or size, we examined ten spinal cords from intact adults of each sex. Cords were frozen sectioned at 50 µm and alternate sections mounted and stained with thionin. The caudalmost section in which the medial and lateral motoneuronal columns were distinguishable was chosen as anchor point; MNs of the medial and lateral clusters were counted for 20 sections rostrally. Counts were also made of the "presplit" cluster for 10 sections caudal to the anchor point. Raw counts were corrected for split nuclei by the method of Konigsmark. Nuclear and soma areas were measured from camera lucida drawings of 12 cells/cluster/animal. There was a sex difference in soma area and raw MN count. However, the latter difference appears to be an artifact of the difference in cell size since the correction procedure eliminated the difference in MN number.

	MEAN SOMA AREA±SEM (µ²)			CORRECTED # MNs±SEM		
Cluster:	Presplit	Medial	Lateral	Presplit	Medial	Lateral
Males	942±44	981±47	969±44	179±14	476±12	407±14
Females	786±44	799±24	813±35	156±8	475±16	402±24

2-way ANOVA shows a sex difference in MN size ($p<.001$) but not # ($p=.81$).

Supported by a NSF predoctoral fellowship (LMF) and March of Dimes (SMB).

380.16

ANDROGEN DOES NOT INCREASE MOTONEURONAL UPTAKE OF CT-HRP. Marianne Leslie and S.Marc Breedlove, Psychology Department, U.C. Berkeley, Berkeley, CA 94720.

It has been suggested that systemic androgen may augment cholera toxin-conjugated horseradish peroxidase (CT-HRP) uptake in hormone-sensitive systems. We tested this possibility by gonadectomizing 38 male rats at 50-56 days of age, and implanting of silastic capsules filled with testosterone (T) or blank (B). 25-44 days later, CT-HRP was injected into 2 muscles: the left side of the hormone-sensitive bulbocavernosus muscle (BC) (2ul), innervated by the spinal nucleus of the BC (SNB) and the right *flexor digitorum brevis* (FDB) (1ul), innervated by the retrodorsolateral nucleus (RDLN). Animals were killed 8-22 hours later, perfused, spinal cords frozen-sectioned at 50u, and motoneurons containing HRP reaction product counted by nucleus and by intensity of labeling (heavy or light).

Mean Total # Cells Labeled (heavy & light, both muscles):

Grp	Hrs: 8	10	13	16	19	22
T	0	12±7	27±8	76±5	80±50	56±32
B	0	26±5	50±13	55±5	99±35	70±5

Two-Way ANOVA: Time $p<.01$, Hormone $p=.4$

2-way ANOVA's of heavily labeled SNB and RDLN motoneurons also reveals a significant effect of time but not hormone.

We found no evidence that systemic androgen could increase the rate of CT-HRP arrival in the spinal cord, discounting one potential source of artifact when using CT-HRP to label dendrites.

380.17

PELVIC AND PUDENDAL NERVES INFLUENCE PACED MATING AND POSTURAL ADJUSTMENT IN FEMALE RATS. M.S. Erskine and E. Kornberg, Dept. of Biology, Boston University, Boston, MA 02215.

The present studies examined the relative contributions of the pelvic and pudendal genitosensory nerves to lordosis behavior, to the pacing of mating stimulation, and to the postural adjustments required for receipt of cervical-vaginal stimulation in estrous female rats during mating. Bilateral surgical transection of the pelvic (Pe), pudendal (Pu), pelvic and pudendal (Pe+Pu) nerves, or sham surgery (Sh) was performed on ovariectomized Long-Evans rats. Lordosis, pacing, and postural adjustment were measured after the sequential administration of estradiol benzoate (EB, 8µg/kg, s.c.) and progesterone (P, 2 mg/kg) in Experiment 1 and after 7 daily injections of EB only (8µg/kg) in Experiment 2. In both experiments, high levels of lordosis behavior were seen in all groups. Pacing of mating stimulation was disrupted in Pe and Pe+Pu groups after EB and P; these females failed to show the pattern of withdrawal from males which in Pu and Sh groups reflected a discrimination between mounts (M) and intromissions (I). After EB only, Pu animals as well as Pe and Pe+Pu failed to show discriminatory responses to M and I. Thus, the pelvic nerves are necessary for the display of pacing behaviors, and normally depend upon the presence of P to become sensitive to selective mating stimulation. Stimulation of the pudendal nerves by males is not sufficient to induce pacing behavior. Postural adjustment, indicated by the ratio M:I, was similar in all groups given EB and P in Experiment 1, but after EB only M:I was elevated in the Pe and Pe+Pu groups. Thus, neither the pelvic nor the pudendal n. are required for postural adjustment if P is present; without P, intact pelvic ns. are necessary for the female to make postural adjustments which increase the likelihood of intromission. HD21802.

380.18

LOCAL SITE OF ACTION FOR DEXAMETHASONE'S CATABOLIC EFFECT ON RAT BULBOCAVERNOSUS. *Mark N. Rand and S. Marc Breedlove*, Psychology Dept., U.C. Berkeley, Berkeley, CA 94720.

We previously found that local treatment of adult rat bulbocavernosus (BC) with testosterone can maintain muscle weight in orchidectomized animals; i.e. steroid capsules placed directly on the muscle show a local anabolic effect (Rand & Breedlove, *Soc. Neurosci. Abstr.* 1987). Last year we reported that the dendritic arbors of motoneurons innervating such T-treated muscles are 37% longer than those of motoneurons innervating the contralateral BC ($p < 0.04$, one-tailed). We now report that local treatment of BC muscle with the synthetic glucocorticoid Dexamethasone (Dex) results in a local *catabolic* effect on the muscle; our analysis of motoneuronal dendritic arbor length is pending.

Gonadally-intact adult male rats 90-180 days of age were implanted with two capsules, one containing Dexamethasone (Sigma), and the other blank. The capsules were sutured on the surface of the muscle, one on one side of the BC, the other on the contralateral side. After 30 days the animals were sacrificed and the BC, spinal cord, and seminal vesicles were dissected out. The left and right BCs were separated and weighed without knowledge of their steroid treatment. Muscle half weights were compared using a repeated measures t-test.

Muscle halves receiving local Dex were 10% lighter than those given blank capsules ($p < 0.003$), indicating that Dex acts at or near the muscle for its catabolic effect.

The effect of this non-androgenic muscle-size manipulation on the length of SNB dendrites should be interesting. If dendrites of motoneurons innervating the Dex-treated muscle half are shorter, they would be shown to vary independently of normal androgen levels. If there is no difference in dendritic length with Dex treatment, then muscle size *per se* would be shown to vary independently of dendritic arbor. We are currently measuring SNB dendrites to test these two possible outcomes.

Supported by NSF #BNS 8451367

381.1

COMMISSURE FORMATION IN SIX MOUSE STRAINS WITH ABSENT CORPUS CALLOSUM. D. Wahlsten and B. Bulman-Fleming[1], Dept. of Psychology, Univ. of Alberta, Edmonton, AB, T6G 2E9 and[1] Depts. of Psychology and Health Studies, Univ. of Waterloo, Waterloo, ON, N2L 3G1 (Canada).

The study sought evidence of single gene effects on forebrain commissure growth. Brains were studied morphometrically in fetuses of 13 inbred and one hybrid mouse strains ranging from 0.2 g to 1.0 g body size. Among six strains known to suffer absence of the corpus callosum (CC) in the adult, there was an association between a) percentage of adults with total CC absence and b) degree of retarded formation of the hippocampal commissure. The rank order of strains on both measures was I/LnJ (worst), 129/J, BALB/cWah1 and a tie of BALB/cWah2, BALB/cByJ and CXBG/By. This association occurs because in these strains the pioneering CC axons usually traverse the midplane via the HC. The cross-sectional area of the medial septal region at the telencephalic midplane was greatly reduced in certain strains (e.g., I/LnJ and BALB/cWah1) but close to normal in others (129/J and BALB/cWah2). These results support previous findings that there are two genetically and developmentally distinct processes which contribute to absent CC (Wahlsten and Smith, J. Hered., 80:11-16, 1989). The causes of CC absence reside not in the CC axons themselves but in either the generation or migration of cells that form the substrate for axon guidance.

Supported by NSERC grant OGP0045825.

381.2

A NEW MOUSE MODEL OF PARTIAL AND COMPLETE AGENESIS OF THE CORPUS CALLOSUM. H.-P. Lipp, R. Waanders* and D.P. Wolfer*. Institute of Anatomy, University of Zürich, CH-8057 Zürich, Switzerland.

Available animal models of callosal agenesis (the mouse strains BALB/c, 129/J and ddN) have the disadvantage of demonstrating a low frequency of this trait. The recently discovered mouse strain I/Ln shows complete penetrance of agenesis of the corpus callosum (personal communication, R.E. Wimer), but has low viability and is only available in small numbers. In order to obtain a complementary mouse model with high callosal variability and high frequency of complete agenesis, we studied the changes in size of the corpus callosum after crossing male I-mice with C57BL/6 females and backcrossing the female offspring.

Changes in midsagittal cross-sectional area of the forebrain commissures were assessed in brains of adult mice (4-8 months of age), 20 per generation. Groups included F1-hybrids (F1), 3 randomly mated generations (F2, F3, F4) and 3 generations from consecutively backcrossing female hybrid mice to the males of the parental strain I/Ln (BX1, BX2, BX3). Area of the corpus callosum changed little in the F2-F4 groups (92-98% as compared to the F1). In the backcross generations, the corpus callosum was increasingly reduced with every generation (BX1: 76%, BX2: 25%, BX3: 5%). This corresponded to the number of mice with apparently complete agenesis of the corpus callosum (BX1: 0 %, BX2: 45% and BX3: 75%). The reduction of the corpus callosum was correlated with a smaller area of the anterior commissure ($r=0.58$, $p < 0.001$) and of the hippocampal commissures ($r=0.86$, $p < 0.0001$), predominantly of their dorsal components.

Thus, variation in size and frequency of agenesis of the corpus callosum can be controlled by means of systematic breeding. Functional and neuroanatomical correlates of callosal variation are studied preferentially in BX2 mice, whereas BX3 mice combine decent viability with a high frequency of complete agenesis of the corpus callosum. Supp. by SNF Grant 3100-009470.

381.3

COMPLETING THE SPLIT IN THE SPLIT-BRAIN RAT: TRANSECTION OF THE OPTIC CHIASM. M.F. Novotny, D.P. Crowne, and I.S. Russell. Dept. of Psychology, Univ. of Waterloo, Waterloo, Ont. N2L 3G1, CANADA.

To study interocular transfer (IOT), we depend on directing sensory input to a single hemisphere. In studies of cat and monkey, division of the optic chiasm routes all visual inflow in monocularly occluded animals to the ipsilateral hemisphere, enabling tests of IOT by section of forebrain commissures. Hemispheric asymmetries have also been investigated in this preparation. In the rat, section of the optic chiasm is formidable, and the alternative has been to exploit the marked asymmetry of crossed and uncrossed projections. Since the small proportion of uncrossed optic fibres in the rat is functional, the role of forebrain commissures in IOT is not firmly established. We describe a stereotaxic technique using a microknife to transect the optic chiasm in the rat that minimizes superficial damage and mortality, and we present illustrative data on the interocular transfer of visual discrimination in chiasm-sectioned and chiasm-plus-forebrain commissure-sectioned animals. Chiasm-sectioned rats are visually competent and show mean savings (75%) on second-eye transfer. Rats with divided chiasm and corpus callosum show no evidence of transfer on first-eye reversal.

381.4

BEHAVIORAL ASYMMETRY IN MALE, FEMALE AND PERINATALLY GONADECTOMIZED RATS. K. J. Schultz. Psychology Department, Univ. of Winnipeg, Winnipeg, MB, Canada R3M 2E9.

Behavioral asymmetry was examined in 317 Sprague-Dawley rats (105 males, 109 females, 103 perinatally gonadectomized males). Adult animals completed four trials on each of seven side preference tasks. Laterality ratios were derived for each task. A direct discriminant function analysis was performed using these laterality ratios as predictors of condition (male, female, gonadectomized male). The discriminant functions derived significantly separated males from females ($p < .000$) but failed to differentiate the two groups of males ($p = .613$). The loading matrix values suggest that the best behavioral predictors for distinguishing between males and females were two circling tasks, a direction choice task and a water bottle preference task. On each of these tasks females exhibited a greater right preference than did males. None of the behavioral tasks discriminated between males and gonadectomized males. Analyses of hemispheric differences in neocortical volume are in progress and suggest that groups differ significantly on these variables.

381.5

SEX DIFFERENCES IN THE ASYMMETRICAL BEHAVIORAL RESPONSE TO BRAIN INJURY IN THE RAT. S.E.Starkstein, J.B.Bryer*, T.H. Moran, R.G.Robinson. Dept. Psychiatry, Johns Hopkins Univ. Sch. Med. Balto., MD 21205.

We have previously reported that right hemisphere or bilateral suction lesions of the dorsal lateral frontal cortex or electrolytic lesions of the nucleus accumbens (NAS) in male rats produce spontaneous running wheel hyperactivity. In the present experiments, we examined these two phenomenon in female rats. Twenty-four adult female Sprague-Dawley rats were given either left, right, bilateral or sham frontal cortical suction lesions and placed in running wheel cages for activity monitoring for 30 days. The results showed no significant between-group differences in locomotor activity, demonstrating, in combination with our earlier findings in male rats, the presence of sexual dimorphism in the lateralized behavioral response to fronto-cortical lesions. In the second series of experiments, 27 adult female Sprague-Dawley rats were given either left, right, bilateral or sham NAS electrolytic lesions and activity was monitored as above. The results showed that rats with either left or right NAS lesions had significantly more locomotor activity than rats receiving either sham treatment or bilateral lesions. These results also contrast with our earlier findings in male rats where right but not left NAS lesions produced significant hyperactivity. Moreover, in adult male rats, bilateral NAS lesions produced more hyperactivity than single right NAS lesions.

381.6

ANTERIOR BRAIN ELECTRICAL ASYMMETRIES IN RESPONSE TO REWARD AND PUNISHMENT. S. Sobotka, R.J. Davidson*, & J. Senulis*. Department of Psychology, University of Wisconsin, Madison, WI 53706.

Recent findings on both brain-damaged and normal individuals indicate differential involvement of the anterior regions of the two hemispheres in approach and withdrawal-related emotional processes. In this study, 15 right-handed subjects were tested in a paradigm which manipulated reward and punishment contingencies while brain electrical activity was recorded. Each trial began with the presentation of a fixation point, which was followed by an arrow which was either in the up or down position. Four seconds later, an imperative stimulus was presented. In the arrow up trials, if the subjects responded sufficiently quickly, they received $.25; in the arrow down trials, if the subjects responded too slowly, they lost $.25. RT criteria were adjusted for each trial block based upon the median RT of the previous trial block. After they responded, subjects were given feedback about their performance. Trial type was randomly varied. EEG was recorded from F3,F4,F7,F8,T3,T4,C3,C4,O1,O2,Pz,Cz and sites between T3 & P3 and T4 & P4, referenced to A1-A2. Power in the theta, alpha, and beta bands of the EEG during the 4 s inter-stimulus interval was computed by Fourier Transformation. In addition, the contingent negative variation (CNV) was quantified during this interval. For alpha power, the Condition X Hemisphere interaction was significant for the frontal leads. Punishment trials were associated with less right frontal power (i.e., more activation) compared with reward trials. These effects were present only in the frontal leads and were more robust when the analysis was restricted to trials on which the subjects won or lost money. No significant Condition X Hemisphere interaction was found for the CNV.

381.7

INVESTIGATION OF LEFT/RIGHT ASYMMETRIES OF SPATIAL NAVIGATION LEARNING IN RATS WITH UNILATERAL 6-OHDA LESIONS OF THE SUBSTANTIA NIGRA. R. Sullivan* and H. Szechtman Dept. Biomedical Sci., McMaster Univ., Hamilton, Ontario, CANADA L8N 3Z5.

Damage to central dopamine (DA) systems in rats impairs aspects of spatial learning in the Morris water maze task. As the right hemisphere in humans is thought to preferentially mediate spatial processing, we sought to determine if selective damage to the left or right nigrostriatal DA system of rats would differentially impair the profile of spatial learning. Male Sprague-Dawley rats (300-350 g) received unilateral 6-OHDA lesions of the left or right substantia nigra (N=24 per group) or control operations (N=14). Group assignment was based on a pretest for directional bias (undrugged) in a 2-m diameter pool, also used for subsequent watermaze testing. Following a rotometer test on apomorphine (APO) at 2 weeks post-lesion, rats were tested for acquisition of a place task (latency to find a submerged platform) in the water maze, for 4 consecutive days. On day 5 (reversal), the location of the platform was switched to the opposite quadrant. Rats received 4 trials a day (water temp. 25° C). Only in reversal, did group differences emerge. Right-lesioned rats were significantly impaired in escape latencies (P=0.016) while left-lesioned rats did not differ from controls. In controls, a leftward pretest bias was correlated with time spent in the former escape quadrant in reversal (r=.56, P=0.018), suggesting right-sided DA dominance for superior retention, a conclusion supported by significant correlations of APO rotation with the same measure in lesioned groups. [Supported by NSERC. HS is a Research Associate of the OMHF].

381.8

SPATIAL DEFICITS AND THEIR LATERALIZATION FOLLOWING UNILATERAL POSTERIOR PARIETAL LESIONS IN THE RAT. S.E. Maier, R.W. Vitols*, M.F. Novotny, & D.P. Crowne, Dept. Psychology, Univ. Waterloo, Waterloo, Ont. N2L 3G1, CANADA.

Both egocentric and allocentric (landmark) cues serve in spatial mapping and navigation. In humans, monkeys, and rats posterior parietal cortex (PPC) is involved in maintaining a reference system to guide spatial movement. Bilateral lesions of PPC yield allocentric deficits. Spatial deficits from unilateral lesions, especially in rats, are less well documented. To show a true homology of PPC function, we tested rats with unilateral lesions on egocentric and allocentric spatial tasks. Rats sustained left or right PPC lesions, lateralized PPC lesions + section of corpus callosum, CC section alone, or sham operations, and were tested on egocentric and allocentric versions of the Morris Water Maze. Both left and right PPC + CC lesions resulted in significant deficits compared to controls on latency and heading error in egocentric place navigation. With reversal of platform location, only controls and animals with left or right PPC lesions were impaired. On the allocentric task, rats with right, but not left, PPC + CC lesions were significantly worse than controls in latency to find the platform. We demonstrate a nonlateralized PPC impairment and a lateralized one in allocentric spatial navigation.

381.9

SOUND LOCALIZATION IN NORMAL AND CALLOSAL AGENESIS HUMAN SUBJECTS. P.Poirier*, S.Miljours*, F.Leporé, L.Richer* and M.Lassonde. Groupe de Rech. en Neuropsy. Exp., Université de Montréal, Mtl, PQ, Canada, H3C 3J7.

In cats, electrophysiological and tracing studies of the primary auditory cortex as well as the corpus callosum suggested the latter's implication in sound localization (Imig et al.,1986; Poirier et al., 1989). The present study examined manual pointing to auditory targets in 4 Ss with callosal agenesis paired to 4 age- and I.Q.-matched controls. Ss were required to locate broadband noise bursts (BBN) at fixed intensity in the horizontal plane in an anechoic chamber. BBNs were delivered randomly through 16 speakers and repeated 5 times. The speakers, which were invisible to the Ss, were mounted at approximately 10° intervals on a perimeter frame having a radius of 50 cm. Ss responses consisted of manual pointing on the frame. Aiming accuracy was assessed by calculating the mean deviation of the pointing responses from the target position. There was no difference between the 2 groups in terms of accuracy. However, acallosal Ss tended to overshoot throughout the field while no such pattern was seen in the control Ss. Furthermore, acallosal and control Ss made significantly more errors when aiming was carried out in the left auditory hemifield. The hemifield differences were not due to sensory deficits in sound localization. This conclusion is supported by visuo-motor pointing task with humans which showed similar differences (Lassonde et al. 1989). (supported by FCAR and CRSNG).

381.10

DEFICITS IN INTERFIELD MEMORY TRANSFER IN CASES OF CALLOSAL AGENESIS. D.J. Ingle, L.S.Jakobson, M.C. Lassonde, & H.C. Sauerwein. Northeastern Univ, Univ.W.Ontario, & Univ. of Montreal.

In man the ease and accuracy with which we remember locations of objects after moving about without vision is taken for granted. When an object is viewed in one hemifield, and the subject rotates so as to "move" the remembered location into the opposite hemifield, does the neural representation of that locus jump from one side of the brain to the other?

We performed a relevant experiment with four subjects with congenital agenesis of the callosum who could point to remembered visual targets (an eye-level cylinder seen for only ½ second) with either hand and in either hemifield. When memory locations remained within the same hemifield all subjects easily discriminated different locations; when the remembered location fell into the opposite hemifield after rotation locations of 30° vs 60° from the midline were very poorly discriminated.

These results support the hypothesis that the underlying memory representations normally do change hemispheres post rotation, since good compensation occurred in controls equated for I.Q.

381.11

EFFECTS OF SEX AND AGE ON REGIONAL MORPHOLOGY OF THE HUMAN CORPUS CALLOSUM. P.E. Cowell, L.S. Allen*, N. Zalatimo*, and V.H. Denenberg. Biobehavioral Sciences Graduate Program, Univ. of Connecticut, Storrs, CT 06269 and Dept. of Anatomy and Cell Biology, UCLA School of Medicine, Los Angeles, CA 90024.

Midsagittal callosal (CC) tracings from MRIs of 73 pairs of age-matched males and females (age 2-79 years) were digitized using computer assisted software. The following parameters were derived: 99 percentile widths along the curved longitudinal axis, length of this axis, area, and perimeter. The width percentiles were grouped into regions based upon prior factor analyses of human callosal material. The regions are widths 3-18 (W3-18), W22-39, W49-62, W65-74, W77-85, W89-94, and W95-99. Trend analyses of these regions with Sex and Age (blocked in 10-year bins) as independent variables, found the following significant effects. The two anterior-most regions (W3-18 and W22-39) had a cubic Sex x Age interaction. In both regions males reached a maximum value at Age 21-30 while females did not peak until Age 41-50. In W3-18 both sexes dropped sharply after peaking. Their callosal widths after age 60 were no greater than seen in childhood. The same phenomenon was seen for females in region W22-39, while male widths remained asymptotic over age. The remaining callosal regions were relatively homogeneous: the CC of both sexes grew with age (quadratic effect) and showed some gradual diminution but no sharp drop off. The genu and anterior body were the CC regions most sensitive to the interactive effects of sex and age.

381.12

CORRESPONDENCE OF AUDITORY AND VISUAL LATERAL ASYMMETRIES FOR LANGUAGE PROCESSING IN DEXTRALS BUT NOT IN NON-DEXTRALS. Z. Caramanos*, R.J. Zatorre and D. Bub*. Montreal Neurological Institute, McGill University, Montreal, Quebec, Canada. H3A 2B4.

Cross-modal correlations of visual and auditory measures of hemispheric language dominance were obtained for 15 dextral and 30 non-dextral human subjects. The auditory measure, ear advantage on the dichotic fused rhymed words test (B. Wexler & T. Halwes, Neuropsychologia, 21:59, 1983) is known to relate to individual hemispheric asymmetry on the intracarotid sodium Amytal test (R. Zatorre, Neuropsychologia, 27:1207, 1989). The visual measure involves the difference between the slopes of mean reaction times to words presented in the left and right visual fields as a function of length, which is taken to reflect hemispheric differences in reading efficiency (D. Bub & J. Lewine, Brain Lang., 33:161, 1988). Both dextrals and non-dextrals showed the expected distributions of asymmetry on the two measures. Dextrals showed a significant correlation (r=.56) between scores on the two tests, suggesting that, to some extent, a common mechanism may underlie the asymmetries observed in the two modalities. Non-dextrals, however, did not demonstrate significant cross-modal correlations (r=.13), which may indicate a partial dissociation between visual and auditory laterality in the non-dextral population.

381.13

HAND AND SEX DIFFERENCES IN SYLVIAN FISSURE MORPHOLOGY S.F. Witelson and D.L. Kigar, Dept. of Psychiatry, McMaster Univ., Hamilton, Ont., Canada, L8N 3Z5

Morphology and asymmetry in the Sylvian fissure (SF) in the human brain was studied by dividing it into 3 segments: anterior (A), horizontal (H), vertical (V). Segment perimeters in each hemisphere were measured in 67 brains (24 men, 43 women) from individuals whose hand preference [consistent-right-handed (CRH) and nonCRH] was tested prior to death. Marked asymmetry was observed: left (L) HSF was greater than right (R); R VSF was greater than L; ASF showed no asymmetry. Sex and Hand were factors in HSF only. (1) Men had greater asymmetry than women. (2) Men had a greater HSF than women but only for L HSF. (3) Only among CRH did men have a greater HSF (over both hemispheres) than women, suggesting that hand has a greater effect in men. In summary, there are two complementary asymmetries in the SF (HSF & VSF). The influence of handedness in men supports the role of SF morphology in functional asymmetry. The sex difference in the association of SF anatomy and handedness is consistent with previous results that area of the isthmus of the corpus callosum varies with hand only in men (Witelson, 1989).

381.14

EMERGING SIGHT-WORD READING IN A VISUALLY AGNOSIC AND PROSOPAGNOSIC CHILD. I.S. Brown and F.B. Wood*. Section on Neuropsychology, Bowman Gray School of Medicine of Wake Forest University, Winston-Salem, NC, 27103.

We report a patient who at 16 months of age suffered cardiac arrest and anoxia during bronchoscopy to remove an aspirated bean. Onset of seizures originating in the left hemisphere occurred soon after. Following the anoxic episode visual evoked response showed functioning of the visual pathways. At age 5, magnetic resonance imaging revealed multiple high signal lesions in both parieto-occipital cortices, worse on the right. Now at age 8, seizures are controlled with Tegretol; visual acuity is decreased, but relatively intact with corrective lenses. His verbal IQ is 74 and verbal short-term memory is within normal limits. He does simple computations mentally, spells orally, reads simple sight words in large print, and is able to name many real objects in his environment, apparently employing color and shape cues to do so. However, with few exceptions he cannot identify large pictures of similar objects, or actual faces of familiar people, including his mother. Dissociation of early reading skills and non-verbal skills in this child is striking and informative, not only by reference to a large normal sample of 8 year olds studied through our NICHD Program project on dyslexia, but also (following Caramazza) as a single case existence proof of emerging reading despite visual agnosia.

381.15

COORDINATED NONINVASIVE STUDIES (CNS) PROJECT. J.L.Lauter. Speech & Hearing Sciences, Univ. of Arizona, Tucson, AZ 85721

Several noninvasive methods for studying human brain structure and function are combined in a test battery to study aspects of behavior in the same subjects. Each individual is tested with behavioral methods, MRI, EPs, qEEG, PET,and MEG. Current focus is on asymmetries for complex sounds. First each subject is trained on dichotic listening for two sound sets which evoke "opposite" asymmetries. Next brain anatomical asymmetries are measured with MRI, and a repeated measures auditory EP series is done to define brainstem asymmetries. Then each individual subject is run on the two sound sets while being monitored with qEEG, then PET, then MEG. Results to date show: 1) good "internal consistency" comparing behavioral, anatomical, and physiological asymmetries within subjects; 2) individual differences in the specifics of the various asymmetries; and 3) agreement across subjects in the patterns of these asymmetry "profiles." Findings suggest that the approach is not only viable, but that exploiting the complementarity of the noninvasive techniques may reveal unsuspected relations among aspects of human neuroanatomy, neurophysiology, and behavior.

DRUGS OF ABUSE: OPIOIDS

382.1

CONDITIONED AND UNCONDITIONED MORPHINE WITHDRAWAL IN THE HAMSTER. P. Schnur. Center for Alcohol and Addiction Studies, Brown University, Providence, RI 02904.

Naloxone-precipitated morphine withdrawal was studied in morphine-pelleted and in subcutaneously injected hamsters. On five days, morphine-pelleted (75 mg) hamsters were injected with naloxone (1 mg/kg) in a distinctive environment. They were observed for signs of withdrawal 10 min before and 30 min after the naloxone injection. Results indicated that a) withdrawal intensity was a direct function of the number of implanted pellets, and b) compared with several control groups, conditioned withdrawal developed among animals withdrawn in the distinctive environment and was evident up to 30 days after pellet removal. To extinguish conditioned withdrawal, some animals were given daily saline injections for 1 wk in the distinctive environment while others remained in the home cage. Conditioned withdrawal was attenuated, but not eliminated by the extinction treatment. A separate experiment indicated that verapamil (10 and 20 mg/kg) did not attenuate naloxone-precipitated withdrawal. In other experiments, withdrawal was precipitated by naloxone (0,0.4, 1 mg/kg) following 4 or 8 sc injections of morphine (15 mg/kg). Withdrawal, measured as above, as well as by the disruption in morphine-elicited running wheel activity, was evident after 4 morphine injections. Again, verapamil failed to attenuate withdrawal. (Supported by NIDA Fellowship and by a Rhode Island Foundation Grant)

382.2

MORPHINE AND COCAINE-REGULATED PHOSPHOPROTEINS (MC-RPPs) IN CRITICAL REWARD REGIONS OF RAT BRAIN: IDENTIFICATION OF TYROSINE HYDROXYLASE (TH) AND OTHER DRUG-REGULATED PHOSPHOPROTEINS. D.B. Beitner and E.J. Nestler, Laboratory of Molecular Psychiatry, Depts. of Pharmacology and Psychiatry, Yale University School of Medicine, New Haven, CT 06508.

The nucleus accumbens (NAc) and ventral tegmental area (VTA) appear to mediate some of the rewarding properties of opiates, cocaine, and other drugs of abuse. In the present study, we have identified a number of phosphoproteins, among which is TH, that are regulated by both chronic morphine and cocaine specifically in these brain nuclei.

Protein phosphorylation was analyzed in brain regions of control rats and rats treated chronically with cocaine or morphine by back phosphorylation with cAMP-dependent protein kinase and 2D-gel electrophoresis. We found that chronic, but not acute, morphine and cocaine treatments regulated a number of the same phosphoproteins in the VTA and NAc. Both treatments increased the total amount of TH in the VTA, but reduced its phosphorylation state (without a change in its total amount) in the NAc. Several other, novel phosphoproteins also showed similar regulation by chronic morphine and cocaine in these brain regions. Drug regulation of most of these various phosphoproteins in the VTA and NAc showed regional specificity in that such regulation was not observed in the substantia nigra and striatum.

We propose that these **M**orphine and **C**ocaine-**R**egulated **P**hospho-**P**roteins (termed "**MC-RPPs**"), together with increased adenylate cyclase and cAMP-dependent protein kinase activities observed in the NAc in response to chronic morphine or cocaine (see Nestler et al., this volume), represent part of the biochemical basis by which these drugs of abuse alter the functional state of brain reward regions.

382.3

A GENERAL ROLE FOR ADAPTATIONS IN G-PROTEINS AND THE CYCLIC AMP SYSTEM IN MEDIATING THE CHRONIC ACTIONS OF MORPHINE AND COCAINE ON BRAIN FUNCTION. E.J. Nestler, D.B. Beitner, M. Hayward,* K.S. Sevarino, and R. Terwilliger. Lab. of Mol. Psychiatry, Depts. of Psychiatry and Pharmacology, Yale Sch of Med, New Haven, CT 06508.

We have shown that chronic morphine increases levels of Giα, Goα, adenylate cyclase (AC), cyclic AMP-dependent protein kinase (cA-K), and certain phosphoproteins (MARPPs) in the locus coeruleus, but not in some other brain regions studied (see *J. Neurosci.* 9:4371, 1989). These changes may occur at least in part at the level of gene expression.

In the present investigation, we surveyed the effects of chronic morphine or chronic cocaine treatments on the G-protein/cyclic AMP system in a large number of brain regions. While most regions showed no regulation in response to morphine, nucleus accumbens (NAc) and amygdala did show increases in AC and cA-K, and thalamus showed an increase in cA-K only. Morphine regulation of G-proteins was variable, with decreased levels of Giα and Goα seen in NAc, increased levels in amygdala, and no change in thalamus. Interestingly, chronic cocaine produced similar changes compared to morphine in G-proteins, AC, and cA-K in NAc, but not in the other brain regions studied.

These results indicate that changes in the G-protein/cyclic AMP system represent mechanisms by which a number of opiate-sensitive neurons adapt to chronic morphine and contribute to varying aspects of opiate tolerance and/or dependence in these cells. The findings that chronic morphine and cocaine produce similar changes in G-proteins, AC, cA-K, and phosphoproteins (see Beitner et al., this volume) in the NAc, a brain region important for the reinforcing actions of abused substances, suggests further that changes in the G-protein/cyclic AMP system may also underlie psychological aspects of drug addiction.

382.4

MORPHINE-INDUCED EFFECTS ON EEG AND BEHAVIOR DIFFER IN LEWIS AND FISCHER 344 RAT STRAINS.

L. Mayo-Michelson,* N.C. Paquette, H. Stamidis,* F.R. George* and G.A Young. Dept of Pharmacol., 20 N. Pine St. Sch. of Pharmacy, Univ. of MD., Baltimore, MD 21201.

Previous studies have demonstrated genetic differences in various inbred strains of rats during preference tests and operant self-administration of ethanol, cocaine and various opiate derivatives (George and Goldberg, 1989). Lewis (LEW) rats self-administered and displayed a preference for these drugs, while Fischer 344 rats did not. In the present study, the effects of morphine (10 mg/kg, i.v.) on EEG and behavior were examined and compared in these two strains. Duration of morphine-induced EEG slow-wave bursts and associated behavioral stupor was greater in LEW rats. Latency to onset of slow-wave sleep after morphine injection was also greater in LEW rats. Effects of morphine on EEG spectral parameters are being assessed. These EEG effects may reflect differences in neurosensitivity and/or opioid receptor populations. (Supported by NIDA DA01050 and NIAAA AA07754).

382.5

MORPHINE-INDUCED HYPOTHERMIA AND SUPPRESSION OF LUTEINIZING HORMONE (LH) SECRETION: A MECHANISM FOR MORPHINE TOLERANCE. R. Ganesan, T. Romano* & J. Simpkins, Dept. of Pharmacodynamics, Univ. of Florida, Gainesville, Florida 32610.

We investigated the role of opponent processes in morphine tolerance by comparing morphine's effects on body temperature and LH secretion. All experiments used 4-week ovariectomized rats which were administered either a low or a high dose of morphine (5 or 30 mg/kg, s.c.) daily for 6 days. The low dose induced hyperthermia and caused a transient suppression of LH levels (for 0.5 h) following the first exposure. Both responses showed little change with repeated injections. The high dose induced hypothermia and a sustained suppression of LH (up to 6 h) following the first exposure. After the sixth injection of 30 mg/kg morphine, rats showed hyperthermia in contrast to the initial hypothermia. With the LH response, tolerance to the high dose was exhibited by an accelerated rate of recovery to normal LH levels rather than an attenuated suppression of LH. These results are best explained by an opponent-process account of morphine tolerance.

Our previous investigations indicate that luteinizing hormone-releasing hormone (LHRH) attenuates morphine's effects on various response systems by inducing responses which oppose those of morphine. The present results are consistent with the hypothesis that enhanced LHRH release may mediate tolerance to morphine. (Supported in part by AG 02021)

382.6

THE NMDA ANTAGONISTS LY274614 AND MK801 AND MORPHINE WITHDRAWAL: A BEHAVIORAL AND ELECTROPHYSIOLOGICAL STUDY. Kurt Rasmussen, Marsha E. Stockton and Paul Ornstein, Lilly Research Labs, Eli Lilly & Co., Lilly Corporate Center, Indianapolis, IN 46285.

There is a clinical need for improved treatments for opiate dependency, especially since intravenous drug abusers are at increased risk for contracting the AIDS virus. This study was designed to evaluate the effects of compounds that are selective for a subtype of excitatory amino acid receptor (the NMDA receptor) on morphine-withdrawal behavior and withdrawal-induced activation of noradrenergic neurons in the locus coeruleus (LC). Systemic administration of the non-competitive NMDA antagonist MK801 dose dependently blocked the behavioral signs of withdrawal in morphine-dependent rats. However, the same doses of MK801 that blocked morphine withdrawal also simultaneously (and dose dependently) produced PCP-like behavioral effects. The competitive NMDA antagonist LY274614 also dose dependently blocked the behavioral signs of withdrawal in morphine-dependent rats but did not produce any PCP-like behavioral effects. Single unit recordings of LC neurons from morphine-dependent animals showed that neither MK801 nor LY274614 blocked the withdrawal-induced activation of these neurons. These results indicate that non-competitive NMDA antagonists like MK801 may not be useful to alleviate opiate-withdrawal symptoms in man because of their PCP-like side effects. However, competitive NMDA antagonists like LY274614 could be of great benefit for alleviating opiate-withdrawal symptoms in man. In addition, NMDA antagonists (unlike the alpha-2 agonist clonidine, which is currently in clinical use) attenuate the behavioral signs of morphine withdrawal without blocking the withdrawal-induced increase of LC unit activity.

382.7

VERAPAMIL REDUCES HYPERCAPNIA AND EUPHORIA PRODUCED BY MORPHINE IN HUMANS. D.B. Vaupel, A. Della-Puppa, W.R. Lange, and E.D. London. NIDA Addiction Research Center, Baltimore, MD 21224.

Verapamil (V), a calcium channel blocker, antagonizes morphine (M)-induced respiratory depression in rats (Szikszay, M. et al., J. Pharmacol. Exp. Ther., 238:192, 1986). As such an antagonism might be useful therapeutically, we extended the earlier work to a study in human volunteers. We tested the ability of V to reduce M-induced respiratory depression and to modulate subjective effects of M in four experienced, male heroin users. Five treatments, each consisting of two, simultaneous 2 min i.v. infusions, included: saline (S) + S; M 10 mg + S; M 10 mg + V 2.5, 5 or 10 mg. Carbon dioxide tension in the blood was measured transcutaneously ($P_{tc}CO_2$), and respiratory rate was determined by impedence pneumography. Questionnaires and visual interval scales were used to evaluate subjective changes. Observations were made from 0.5 hr before to 4 hr after drug administration. M significantly increased $P_{tc}CO_2$ levels above S + S levels ($P < 0.05$), whereas none of the increases in $P_{tc}CO_2$ following M + V combinations differed from S + S condition. No consistent effects on respiratory rates were found. M produced slight to moderate subjective effects, which included self-reports of "good effects", "liking" and of being "high." These marginally significant effects ($P < 0.1$) occurred in 3 of 4 subjects. Additionally, all three doses of V consistently reduced the positive effects of M on mood, as well as the strength of the drug effect. Although further studies are indicated, the present results suggest that co-administration of calcium channel antagonist drugs, such as V, may improve safety and reduce abuse liability of M treatments.

382.8

ANALOG OF FMRF-NH_2-LIKE MAMMALIAN PEPTIDE ATTENUATES MORPHINE ABSTINENCE SYNDROME. J.R. Lake, D.H. Malin, M.V. Hammond*, S.L. Brown*, J.L. Sims*, K.R. Arcangeli*, G.M. Moore* and K. Payza. Univ. of Houston-Clear Lake, Houston, TX 77058 and NICHD, Bethesda, MD 20892.

It has been suggested that the peptide FLFQPQRF-NH_2 (F-8-F-NH_2) may play a role in opiate dependence and subsequent abstinence syndrome. This peptide precipitated opiate abstinence syndrome, while IgG from antiserum against this peptide largely prevented subsequent naloxone-precipitated abstinence signs in dependent rats. The peptide desamino-YFLFQPQR-NH_2 (daY-8-R-NH_2) was synthesized as a possible F-8-F-NH_2 antagonist. It appears to be a mixed agonist/antagonist. At doses of 2μg or higher it precipitates abstinence syndrome in dependent rats in a manner similar to F-8-F-NH_2. However, 600ng i.c.v. did not induce withdrawal and attenuated subsequent naloxone-precipitated withdrawal. Fourteen rats were cannulated in the 3rd ventricle and rendered dependent by 7 days continuous s.c. infusion of morphine sulfate (0.3 mg/kg/hr. via Alzet osmotic minipump). Seven rats were injected with daY-8-R-NH_2 i.c.v. while 7 received saline alone. Twenty-eight mins. later, all rats were challenged with 10μg naloxone through the same cannula. During the following 20 mins., each rat was observed under "blind" conditions for standard abstinence signs. The daY-8-R-NH_2-pretreated rats had 80% fewer abstinence signs than saline-pretreated controls, a significant difference, $p<.001$. There were significant differences in writhes/gasps, shakes/tremors, teeth chatter/chewing and ptosis.

382.9

BUPRENORPHINE-INDUCED TOLERANCE TO DISCRIMINATIVE STIMULUS EFFECTS OF MORPHINE AND BUPRENORPHINE. G. Kapitsopoulos* and A.M. Young. Department of Psychology, Wayne State University, Detroit, MI 48202

The effects of acute or repeated treatment with buprenorphine (BUP) were examined in Sprague-Dawley rats trained to discriminate saline and 3.2 mg/kg morphine (MS) under a two lever fixed-ratio 15 schedule of food delivery. Cumulative doses of MS (0.1-3.2 mg/kg) or BUP (0.001-0.32 mg/kg) evoked MS-appropriate behavior in a dose-dependent manner, with complete generalization at 1.78 mg/kg MS or 0.032 mg/kg BUP. Acute doses of BUP given 30 min (0.003-0.01 mg/kg) or 24 h (0.1-0.3 mg/kg) before a test decreased the dose of MS or BUP required for stimulus control in an additive manner. In contrast, suspending training and administering repeated doses of BUP (0.32 mg/kg b.i.d.) for 1 to 2 weeks increased the dose of MS or BUP required for stimulus control by at least 3-fold. However, during repeated BUP treatment, MS did not evoke stimulus control in 3 of 8 rats, and BUP did not evoke MS-like stimulus control in 2 of 8 rats. Repeated BUP treatment increased the dose of MS required for rate suppression by >6-fold, and that of BUP by >30-fold. Stimulus control by BUP recovered within 1 week after BUP treatment ended; stimulus control by MS, within 2 weeks. These results suggest that repeated treatment with BUP produces a prolonged tolerance to the MS-like stimulus effects of MS and BUP. (Supported by USPHS grants DA03796 and K02 DA00132.)

382.10

HEROIN SELF-ADMINISTRATION ON A PROGRESSIVE RATIO SCHEDULE OF REINFORCEMENT: THE EFFECT OF DOSE MANIPULATION, OPIATE ANTAGONISM AND SALINE REPLACEMENT. S.A.L. Bennett and D.C.S. Roberts Department of Psychology, Carleton University, Ottawa, Ont., K1S-5B6.

Heroin self-administration was examined using a progressive ratio schedule of reinforcement. On the first test day, the response requirements began at 1 and escalated exponentially after each drug infusion. On the second and subsequent test days, the initial response requirements were adjusted according to the previous day's final ratio. An inverted U dose response relationship was established for animals self-administering 12.5, 25, 50, and 100 µg/injection heroin with breaking points peaking at the 50 µg/injection dose. Breaking points were shown to significantly increase following saline substitution for heroin injection and remain elevated for several days before extinction of the self-administration response was observed. Heroin-reinforced breaking points were shown to decrease rapidly following daily IP naltrexone (0.6 mg/kg) pretreatment. These results suggest that progressive ratio schedules of reinforcement are useful in quantifying changes in opiate-motivated behaviour (Supported by the M.R.C).

382.11

ULTRASONIC VOCALIZATION AS A MEASURE OF LONG-TERM AND SHORT-TERM TOLERANCE AND WITHDRAWAL IN NEONATAL RATS. C. P. Cramer, R. A. Fite*, and M. S. Fanselow. Dept. of Psychology, Dartmouth College, Hanover, NH 03755.

Ultrasonic vocalizations are typically reduced by acute adminstration of opiates (eg., Kehoe & Blass, 1986). We compared this behavior to nociceptive responses (pawlift on a hotplate) and used it as an index for studying the development of tolerance to and withdrawal from opiates in neonatal rats.

Morphine sulfate (2, 4, or 10 mg/kg) given once daily for the first 10 postpartum days essentially eliminated vocalizations compared to saline controls on each day. On Day 11, pups from each treatment group were adminstered saline, morphine (1 mg/kg) or naloxone hydrochloride (10 mg/kg). The morphine-exposed groups were as vocally depressed by the test injection of morphine as was the saline control group. Naloxone did not increase vocalization in the drug-exposed groups. Thus, unlike adults, neonatal rats did not develop tolerance and withdrawal using what have been termed "long-term" parameters. An alternative form, "short-term" tolerance, has been proposed for rats given closely-spaced doses of opiate (Baker & Tiffany, 1985). To test this form, pups received morphine (10 mg/kg) or saline at 6-hr intervals for 2 days, beginning on Day 1 or Day 10. They were then given saline, morphine (1 mg/kg) or naloxone (10 mg/kg) 6 or 36 hr after the last exposure and tested using ultrasonic vocalization and pawlift latency. In general, newborn rats appeared to be capable of acquiring tolerance and withdrawal if given massed injections of morphine in high doses. The apparent distinction between infant rats' ability to form long- and short-term tolerance suggests that the mechanisms surbserving each form of tolerance develop at different ages.

382.12

THE OBLIGATORY NATURE OF MORPHINE-INDUCED ORAL STEREOTYPIES. J. Pollock, R. Galli* and C. Kornetsky. Laboratory of Behavioral Pharmacology, Boston Univ. Sch. of Med., Boston, MA 02118.

Three high doses of morphine will produce oral stereotypies in rats that can be re-expressed up to 6 months later. Because amphetamine-induced oral dyskinesias are often dependent on the mode and setting of drug delivery, the present study determined the role of these variables in morphine-induced stereotypy. Male F-344 rats were continuously infused with morphine (53 mg/ml) over 36 hours, or were injected with 3 high doses of morphine (10,20,20 mg/kg, sc) over 24 hours. Throughout the morphine administration, rats were placed in test chambers with steel bar floors. Thirty days later animals received morphine (4.0 mg/kg) and were observed for evidence of oral stereotypy. There was no significant difference in the incidence of stereotypy between the two groups. In a second study rats received 3 high doses of morphine and were placed in either the test chambers with floor bars or in a solid plastic box. Thirty days later, all animals were injected with morphine (4.0 mg/kg) and were placed in the chambers with bar floors. All animals showed oral stereotypy. Unlike amphetamine-induced oral dyskinesias, the stereotypies produced by morphine appear to be obligatory. (Supported by NIDA grant DA02326 and RSA DA00099 to CK).

382.13

THE ROLE OF NIGRAL OPIOID RECEPTORS IN MEDIATING MORPHINE WITHDRAWAL. M. Nagy and A. A. Baumeister. Department of Psychology, Louisiana State University, Baton Rouge, LA 70803.

These studies were conducted to investigate the role of opioid receptor subtypes in the substantia nigra in behavioral symptoms of morphine withdrawal. Male Sprague-Dawley rats were given daily injections of increasing doses of morphine sulfate (20-80mg/kg/5ml, s.c.), dissolved in a sustained release preparation (7.5% mineral oil:42.5% Arlacel A:50% saline). Eighteen hours after the last morphine injection, animals received bilateral intranigral injections of 5nm/0.5µl, naloxone (nonspecific antagonist), beta-FNA (mu specific antagonist), nor-BNI (kappa specific antagonist), or sterile water (0.5µl). Withdrawal behaviors were observed for 20 minutes following intranigral injection. Naloxone produced significant increases in wet dog shakes, ptosis, teeth chatter and squealing (irritability to touch), when compared to the intranigral water control. The only effects produced by beta-FNA and nor-BNI were an increase in teeth chattering and wet dog shakes, respectively. Ptosis and squealing were significantly increased by naloxone compared to beta-FNA and nor-BNI. These data suggest teeth chattering and wet dog shakes in morphine dependent rats are mediated by nigral mu and kappa receptors, respectively, whereas squealing and ptosis are not mediated exclusively by these receptor subtypes.

382.14

Electrophysiological identification of Accumbens (NAS) neurons by evoked responses to multiple afferent stimulation explains variance of NAS opioid drug effects. R. L. Hakan* and S.J. Henriksen, *U. of North Carolina-Wilmington & Research Inst. of the Scripps Clinic, La Jolla, Ca. 92037

The effects of systemic morphine on fimbria-evoked NAS neuronal responses in anesthetized rats have been described as heterogeneous (Hakan & Henriksen, 1987; Neursci. Lett). As part of ongoing studies of the effects of opiate drugs on NAS neuronal activity we currently report that the effects of systemically administered opiates on ventral pallidum (VP)-evoked NAS neuronal activity are also heterogeneous. However, concurrent stimulation of VP and fimbria input to the NAS have shown patterns of convergence and nonconvergence that indicate several different categories of NAS neurons, and has consequently provided a means of functionally identifying distinct NAS neuronal subpopulations (Hakan & Henriksen, 1989). Further analysis of morphine effects in this light has indicated that, in contrast to earlier observations of heterogeneous opiate actions, for some NAS subpopulations morphine effects are largely uniform. For example, those NAS neurons that are monosynaptically driven by both fibria and VP stimulation are consistently inhibited by systemic morphine. Yet the effects of opiates on other NAS subpopulations are still apparently mixed. It is predicted that further functional categorization of these subpopulations by reponses to other afferent inputs will ultimately explain the remaining variance of opiate effects in this region.

382.15

BLOCKADE OF MORPHINE INDUCED STIMULATION OF MESOLIMBIC AND STRIATAL DOPAMINE RELEASE BY IBOGAINE. I.M.Maisonneuve, R.W.Keller, Jr. and S.D.Glick. Dept. Pharmacology & Toxicology, Albany Medical College, Albany, NY 12208.

Ibogaine, an indolalkylamine, has been claimed to abolish the drug craving of a few heroin and cocaine addicts for at least 6 months (patent No. 4,449,096) and, in preliminary studies in this laboratory, one to five doses of ibogaine (40 mg/kg) has decreased I.V. morphine self administration by rats for several days thereafter. Drugs of abuse belonging to different pharmacological classes have been shown to stimulate dopamine (DA) transmission in the mesolimbic system; this is believed to be the basis for the rewarding effects of those drugs. The present study used the microdialysis technique to determine the effects of a single dose of ibogaine on basal extracellular DA levels and also the effects of ibogaine pretreatment on morphine stimulation of brain DA systems. Ibogaine (40 mg/kg, IP) decreased DA extracellular levels in nucleus accumbens and striatum and increased DA levels in the prefrontal cortex. When injected 19 hr prior to a morphine challenge (5 mg/kg, IP), ibogaine (40 mg/kg, IP) prevented the rise in DA levels in the striatum and nucleus accumbens observed after morphine injection alone. These results are consistent with the proposed use of ibogaine in the treatment of opiate addiction. (Supported in part by NDA International, Inc. and NIDA grant DA03817).

382.16

MORPHINE ACTS IN THE PARABRACHIAL NUCLEUS TO PRODUCE DISCRIMINATIVE EFFECTS. T.V. Jaeger* and D. van der Kooy. Dept. of Anatomy, Univ. of Toronto, Toronto, Canada, M5S-1A8.

Previous experiments have shown that the motivational properties of morphine that support conditioned taste aversion and place preference are distinct from those which underlie its discriminative stimulus actions. However, the neural basis for this dissociation is not yet apparent. We attempted to identify the neuroanatomical sites that mediate the discriminative stimulus properties of morphine using a discriminated taste aversion paradigm. Rats were injected i.p. with 5 mg/kg of morphine 15 min prior to the presentation of a 0.1 % saccharin solution. After 20 min of exposure to the flavour, lithium chloride (130 mg/kg i.p.) was injected. On alternate days, an injection of 0.9% saline both preceded and followed the presentation of saccharin. Animals learned to consume significantly less saccharin after morphine than after saline injections. Guide cannulae were then implanted into brain areas containing relatively high densities of opiate binding sites, including the hippocampus, medial prefrontal cortex, ventral tegmental area (VTA), nucleus accumbens, dorsolateral striatum, ventromedial thalamus, parabrachial nucleus and the caudal aspects of the periaqueductal grey. Generalization to central routes of administration was then evaluated following the microinjection of morphine (5, 10 and 20 µg) into these brain areas. Dose-dependent stimulus effects like those of systemic morphine were produced in all rats by the administration of morphine into the parabrachial nucleus. Control data showed that these effects were not due to unconditioned effects of central morphine on fluid consumption. Morphine injected into the VTA, the nucleus accumbens and the ventromedial thalamus produced some morphine-appropriate responses in some of the animals tested. In the remaining brain areas, morphine generalized to the systemic saline condition. The data point to ascending visceral pathways (parabrachial nucleus and ventromedial thalamus) and possibly ascending dopamine projections (VTA and nucleus accumbens) as possible substrates for opiate discriminative effects. These putative discriminative substrates are distinct from the descending medial forebrain bundle pathways mediating the positive reinforcing effects of opiates.

382.17

INCREASED D1 AND D2 RECEPTOR ACTIVITY DURING OPIATE WITHDRAWAL: BEHAVIORAL AND BINDING EVIDENCE. J.W. Tidey and K.A. Miczek. Dept. of Psychology, Tufts University, Medford, MA 02155.

The motor and social-aggressive activity of mice is greatly affected by morphine withdrawal; converging evidence particularly implicates altered dopaminergic activity in these behavioral effects. Previously, we reported that the administration of d-amphetamine to morphine-withdrawn mice results in high levels of aggression which outlast motoric withdrawal signs. We have investigated this effect further by administering the selective D1 and D2 agonists SKF 38393 (1-100 mg/kg) and quinpirole (0.1-1 mg/kg) to mice which had undergone removal of s.c. morphine or placebo pellets at varying time points before evaluation of both motor activity and aggressive behavior toward an intruder. The D1 agonist SKF 38393 decreased aggressive behaviors in both morphine-withdrawn and placebo mice without affecting rearing, walking or grooming. The D2 agonist quinpirole maintained elevated levels of attack, threat, and motor behavior in morphine withdrawn mice while all of these behaviors were suppressed in placebo controls. A similar behavioral profile was seen in mice who were administered 3.0 mg/kg SKF 38393 before quinpirole injection. To determine whether these behavioral changes are due to upregulation of dopamine receptors during withdrawal, D1 and D2 binding of 3H-raclopride and 3H-SCH 23390 was measured *in vivo* in the striatum, olfactory tubercles and cerebellum of mice which had undergone morphine or placebo pellet removal 5, 48, or 96 hours previously. Morphine-withdrawn mice showed high levels of D1 and D2 receptor binding as compared to placebo controls; this increased D1 and D2 receptor occupancy during withdrawal may be relevant to the heightened aggressive behavior.

382.18

SOMATODENDRITIC RELEASE OF DOPAMINE IN THE VENTRAL TEGMENTAL AREA FOLLOWING MORPHINE. M.A. Klitenick and P.W. Kalivas. Department of VCAPP, Washington State University, Pullman, WA 99164-6520.

A number of recent studies have suggested a modulatory role for opioids and GABA in the mesolimbic A10 (VTA) dopaminergic system. Opioid injection into the VTA elicits an increase in dopamine (DA) metabolism and locomotor activity, the latter of which can be blocked by DA antagonists. Repeated administration of morphine results in a progressively augmented behavioral response in addition to a decrease in DA utilization within the VTA. GABA has also been shown to regulate DA transmission. Injection of a $GABA_A$ agonist into the VTA activates the mesolimbic DA system, while a $GABA_B$ agonist inhibits DA activity.

In order to further assess the suggested modulatory role of opioids and GABA on the mesolimbic DA system, in-vivo dialysis was used to measure DA and its metabolites in the VTA following administration of morphine (1 and 10 µM) or baclofen (1 and 10 µM) through the dialysis probe. Administration of baclofen through the probe had no effect on DA levels within the VTA; however, morphine was found to elicit a dose-dependent increase in the somatodendritic release of DA, DOPAC and HVA.

382.19

SELF-ADMINISTRATION OF MORPHINE, [D-ALA^2,N-ME-PHE^4-GLY^5-OL]-ENKEPHALIN (DAGO), AND [D-PEN^2,D-PEN^5]-ENKEPHALIN (DPDPE) INTO THE VENTRAL TEGMENTUM OF THE RAT. D. P. Devine and R. A. Wise. Center for Studies in Behavioral Neurobiology, Department of Psychology, Concordia University, Montreal, Canada H3G 1M8.

Intracranial self-administration of opioid agonists which are selective for the µ and ∂ receptor subtypes was demonstrated in independent groups of male Long-Evans rats. Each animal was unilaterally implanted with a chronic guide cannula and was allowed to lever-press for ventral tegmental area (VTA) injections of one of three compounds: morphine (mixed agonist), DAGO (selective µ agonist), or DPDPE (selective ∂ agonist). Each compound was effective in establishing and maintaining a lever-pressing habit in animals with VTA cannulae. Animals with dorsal control cannulae did not learn to lever-press. These results raise the possibility that µ and ∂ receptors in the VTA are involved in the rewarding effects of opiates.

382.20

MORPHINE WITHDRAWAL SYNDROME AFTER LOCAL ADMINISTRATION OF METHYLNALOXONIUM IN SEVERAL BRAIN STRUCTURES. R. Maldonado*, L. Gold, L. Stinus and G.F.Koob. Dept. of Neuropharmacology, Research Institute of Scripps Clinic, La Jolla, CA 92037

Opiate withdrawal is a common occurrence in human opiate addicts that is not life threatening, but is thought to contribute to the maintenance of opiate dependence. Little is known about the neural substrates for the physical signs of opiate withdrawal, although recent work has implicated the region of the nucleus accumbens in the motivational aspects of opiate withdrawal. The purpose of this study was to establish a protocol of morphine withdrawal with minimal between subject variation in order clearly to investigate the neuroanatomical regions implicated in the expression of the physical signs of morphine withdrawal. Morphine withdrawal was precipitated by local administration of methylnaloxonium (MN), a hydrophilic opiate antagonist, into several brain regions in the rat. Cannulae were implanted into the lateral ventricle (ICV), periaqueductal gray (PAG), medial thalamus (MT) and nucleus accumbens (NAC). Following surgery, rats were made physically dependent by sc implantation of two 75 mg morphine pellets. MN (500 ng) was administered to rats with ICV cannulae 60 h after pellet implantation, and was repeated each 12 h until a total of 5 injections was made. MN administered ICV induced a withdrawal syndrome similar to systemic naloxone. Several signs as diarrhea, salivation, lacrimation and rinorrhea did not appear suggesting possible peripheral mediation.The withdrawal syndrome observed after the four last MN injections was similar, showing that it is possible to induce this syndrome several times in the same animals. When MN was injected, 72 h after pellet implantation, into the PAG (250 or 1000 ng) or into NAC (64 or 250 ng) it produced fewer withdrawal signs than when injected ICV (64-4000 ng). Rats injected with MN (64 or 250 ng) into the MT showed almost no signs of withdrawal. These preliminary results suggest that in contrast to the aversive stimulus effects of opiate withdrawal the neuroanatomical substrate for the physical withdrawal signs in the rat seems to depend on multiple brain structures.These results have important implications for our understanding of the basic brain mechanisms involved in opiate dependence, a condition which causes widespread profound human suffering. These studies were performed under protocol ARC 44FEB0 approved by the Institutional Animal Care Committee of the Research Institute of the Scripps Clinic.

383.1

CHF-146 STRAIN DYSTROPHIC HAMSTERS (DH) AS A MODEL FOR MUSCULAR DYSTROPHY WITH CARDIAC HYPERTROPHY. P. L. Johnson, C. I. Tillilie*, T. A. Adamec*, D. R. Shanklin*, and S. K. Bhattacharya. Depts. of Surgery, Anatomy & Neurobiology, and Pathology, University of Tennessee, Memphis, TN 38163.

Membrane-mediated excessive intracellular Ca accumulation (EICA) is a fundamental pathogenetic event in muscular dystrophy (Bhattacharya & Johnson, Neurology India, 37:145, 1989). Concommitant to EICA, elevated plasma CK, histopathology, cardiac dysfunction, and reduced lifespan are common in Duchenne muscular dystrophy (DMD). We have shown striking similarities in pathobiology between DMD and BIO-14.6 strain DH (Muscle & Nerve, 10:168, 1987). Since BIO-14.6 DH are no longer available, we investigated CHF-146 strain male DH from Canadian Hybrid Farms, Nova Scotia, as a model for DMD. CHF-148 strain albino normal hamsters (NH) served as controls. EICA, measured by atomic absorption spectroscopy, was profound in the cardiac and skeletal muscle of DH. Plasma CK and relative cardiac enlargement were also elevated. Among the most noticeable histologic changes in DH were the abundance of atrophic, hypertrophic, and necrotic fibers with fatty infiltration, centronucleation, fascicular disarray, and fibrosis. EM of myocardium revealed mitochondrial Ca deposition and loss of striations. Increased mortality in DH ensues at 90 days of age with average life expectancy of 250 days, compared to 3 years for NH. We conclude that CHF-146 strain DH is an excellent model for therapeutic scanning in DMD. Supported by MDA and NIH Grant AR-38540.

383.2

EVOLUTION OF TREMOR IN A NEUROLOGICAL MUTANT OF SPRAGUE-DAWLEY RATS. Doger E., Vega J*, Holmgren B.* and Vega-Saenz de Miera E.* Dept. de Ciencias Fisiológicas ICUAP, Univ. Autónoma de Puebla. P. O. Box 406, Puebla, México.

In the rat colony of our laboratory, a neurological mutation appeared, characterized by tremor, ataxia, tonic immobility episodes and paralysis. The initial neurological sign is a fine tremor in the tail and hindlimbs which is visible at 1 month age. this syndrome is transmitted by autosomal recessive inheritance.

Tremor frequency was recorded in rats from 15 days to 12 months old, using power spectral analysis following a procedure described by Shinozaki (Neurosc. Res. 2:63-76, 1984). One month old rats exhibited tremor at an average frequency of 7 ± 0.3 Hz; at the age of 2 months and until 12 months the main peak frequency is at 5.9 ± 0.4 Hz. Tremor is attenuated by the administration of the dopaminergic agonist apomorphine (APO) (0.01-0.1 mg/Kg), effect which is not observed with higher doses of APO (0.5-1) nor with the dopaminergic antagonist sulpiride (30-100 mg/Kg). The underlying mechanisms of this hereditary neurological syndrome is under current study by our group.

383.3

CONTRIBUTIONS OF GENOTYPE TO CEREBELLAR MORPHOLOGY: DIFFERENCES IN PRIMARY FISSURE DISTORTION IN INBRED STRAINS OF RATS . G.R. Ward, C.R. Goodlett, J.M. Nichols,* and J.R. West. Dept. of Anatomy, University of Iowa, Iowa City, IA 52242.

Aberrant development of the primary cerebellar fissure in rats, including fusion in the depths of the fissure and the presence of ectopic neurons, has been reported in a number of studies of outbred rats. Substantial variation in the frequency and extent of such distortion has been found both within and between outbred strains. In this study, we examined the primary fissure of eight inbred rat strains--ACI, Brown Norway [BN], Buffalo [BUFF], Fisher 344 [F344], Maudsley Reactive [MR], Marshall [M520], White Norway [WN] and Wistar-Kyoto [WKY]. All subjects were derived from a breeding colony maintained in our laboratory, originally established using breeding pairs obtained from NIH. Sagittal sections, either 30-µm frozen sections or 2-µm JB-4 sections, were taken through the cerebellum, stained with a Nissl stain, and examined microscopically. Several strains [BN, F344, MR] exhibited severe distortions of the primary fissure within the vermis, with extensive fusion of the fissure and numerous ectopic cell clusters. Others [ACI, M520, BUFF] had mild to moderate distortion, usually limited to partial fusion of the primary fissure, with only occasional ectopias. Little or no distortion of the primary fissure was seen in the remaining two strains [WN, WKY]. Regardless of strain, these perturbations were generally more pronounced in the 10-day-old subjects than in adults. A number of other differences were found in cerebellar structure, ranging from ectopic cells in lobule IX [M520] to dramatically increased gyrification [BN]. Although variation in the extent of distortion was observed within strains, the marked individual differences in severity were predominantly accounted for by differences among strains. These findings demonstrate differences among inbred strains in the morphological development of the cerebellum, and suggest that studies of inbred rats may provide a useful model to evaluate the contribution of genotype to aberrant development of cerebellar fissures and lobules. (Supported by Grant #AA 07313)

383.4

TESTS OF GENETIC ALLELISM BETWEEN FOUR INBRED MOUSE STRAINS WITH ABSENT CORPUS CALLOSUM. D.J. Livy and D. Wahlsten, Department of Psychology, University of Waterloo, Ontario, N2L 3G1 and Department of Psychology, University of Alberta, Edmonton, Alberta, T6G 2E9 (Canada).

If two inbred strains show absence of the corpus callosum (CC) because they have the same genetic defect, then a hybrid cross between them should also have CC absence. If the hybrid is normal, the parent strains must differ at one or more relevant genetic loci. Over 450 weanling mice were obtained from five inbred strains and 11 hybrid crosses, and their brains were sectioned sagittally and stained for myelin. All crosses with the normal strain C57BL/6J were normal, demonstrating recessive inheritance. When the I/LnJ strain (100% total CC absence) was crossed with less severely affected strains (BALB/cWah1, BALB/cWah2, 129/ReJ), hybrid mice with a BALB/c parent showed numerous CC defects, whereas those with a 129 parent had less severe defects than 129. Crossing either BALB/c strain with 129 yielded offspring with no or very few CC defects. Crossing the two BALB/c strains produced mice resembling BALB/cWah2. It is concluded that the strains BALB/cWah1, BALB/cWah2 and I/LnJ have essentially the same genetic abnormality, differing only in severity of the defective alleles, but that 129/ReJ differs from these three strains, most likely at a single locus.

Supported in part by an NSERC postgraduate scholarship to DJL and operating grant to DW. Thanks to Kathryn Blom for doing the histology.

383.5

THE TWITCHER MOUSE: IN VITRO STUDIES OF SCHWANN CELLS (SC) ON ADHESIVENESS AND RATE OF PROLIFERATION. A. Komiyama* and K. Suzuki. Department of Pathology, School of Med. Univ. of North Carolina, Chapel Hill, NC 27514.

A previous study of isolated SC from twitcher revealed apparent differences in the rate of cell growth between suckling and adult mice (Irino and Suzuki, 1990). To elucidate the cause of such differences, we examined adhesiveness and rate of proliferation of SC in vitro. SC were isolated from sciatic nerves and brachial plexus of three genotypes (twi/twi, twi/+, +/+) at P10 (group 1) or P30 (group 2) and were cultured (3×10^4 cells/12mm coverslip). Cell numbers were counted and [^{3}H] thymidine-uptake of the SC were determined at 1, 2, 4, 6, and 8 days in vitro (DIV). SC adhesiveness at 1DIV in group 1 was similar in all genotypes and was significantly higher than that from twi/+ and +/+ in group 2. In contrast, SC adhesiveness in twi/twi in group 2 was higher than any genotype in group 1. Thymidine incorporation was generally higher in group 1 than group 2. However, labelling index in twi/twi was less than twi/+ or +/+ in both groups. In twi/twi in group 2, SC adhesiveness was most pronounced but thymidine incorporation remained low. The results indicate that 1) hypofunction or dysfunction exists in twi/twi SC and is more prominent in the adult; and 2) the apparently accelerated cellular proliferation in adult twi/twi is a result of increased adhesiveness of the SC secondary to demyelination in adult twi/twi nerves.

383.6

CHANGES OF NEUROPEPTIDES IN SPINAL CORD AND BRAINSTEM OF WOBBLE MOUSE AT DIFFERENT STAGES OF MOTONEURON DISEASE. K.K.L.Yung*, F.Tang*[1], L.L.Vacca-Galloway. Depts. of Anatomy and Physiology[1], Fac. of Med., Univ.of Hong Hong, Hong Kong.

As a model for study of human motoneuron disease, the autosomal recessive mutant Wobbler mouse (wr) exhibits a progressive loss of motoneurons in spinal cord and brainstem. In early stages of the disease, axons containing substance P (SP) and thyrotropin releasing hormone (TRH) but not leucine enkephalin (LE) appeared increased by immunocytochemistry around cervical motoneurons (Vacca-Galloway, L.L.and Steinberger,C., J. Neurosci. Res., 16:657-670,1986). Recent radioimmunoassay (RIA) studies have shown that TRH, LE and methionine enkephalin (ME) contents increased in the spinal cord at different stages of the wr disease (Tang, F. *et al*, Brain Res., in press, 1990).

In the present study stages of the motoneuron disease were evaluated in NFR/wr mice (NIH, Besthesda, Md.), 3 weeks to 11 months old, identified by behaviorial tests (Lange, D.J. *et al*, J. Neurol. Sci., 61:211-216, 1983). The wr mice (Stages 1 to 4) and their control littermates (Stage 0) were decapitated, the cervical spinal cord, brainstem, also hypothalamus and midbrain were collected, and the TRH, SP, LE and ME contents were compared by RIA.

In experiments wherein all control values were pooled, TRH in wr spinal cord was increased significantly in Stages 1 and 2, SP increased in Stages 1 and 4, and LE increased throughout Stages 1 to 4. When control littermates were statistically pair-matched with wr littermates, SP contents at Stage 1 showed no increase in spinal cord. ME contents increased in Stages 1 and 2 but not 3 and 4 when control values were pooled, but showed opposite results when control and wr values were pair-matched. In wr brainstem TRH increased significantly in Stages 2 and 3, however SP showed no increase. ME and LE became increased in Stages 3 and 4. Trends observed for these peptides in spinal cord and brainstem may relate to disease development.

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383.7

ABERRANT DEVELOPMENTAL REGULATION OF TYROSINE HYDROXYLASE EXPRESSION IN TOTTERING AND LEANER MOUSE MUTANTS. E.J. Hess and M.C. Wilson, Dept. of Neuropharmacology, Research Institute of Scripps Clinic, La Jolla, CA 92037.

The inherited autosomal recessive mutations, tottering (gene symbol: *tg*) and leaner (gene symbol: tg^{la}) are functional alleles of a single gene locus in mice. The tottering mouse mutant exhibits spontaneous absense seizures, focal motor seizures and mild hindlimb ataxia while the congenic mouse mutant leaner displays severe ataxia and no focal motor seizures. We have previously reported ectopic expression of tyrosine hydroxylase (TH) in the cerebellar Purkinje cells of the adult mouse mutants tottering and leaner (Soc. Neurosci. Abstr. 15:986, 1989). In order to assess the onset of ectopic TH expression, TH mRNA expression was examined by in situ hybridization in postnatal day 1 (P1) through P28 tottering and leaner mouse mutants and age-matched heterozygous littermates. Hybridization of the ^{35}S-cRNA TH probe was detected in the locus coeruleus and substantia nigra in all mice from P1 through adulthood. TH expression in the Purkinje cells of tottering and leaner mice was first observed at P18 and persisted through adulthood. On P18, TH expression was also observed in the Purkinje cells of the heterozygous control mice and in wild type C57Bl/6J mice; the TH expression in Purkinje cells of control mice persisted through P28 and was absent in adult mice. These data indicate that TH is normally transiently expressed in Purkinje cells during postnatal development, similar to transient developmental TH expression observed in other brain regions, such as the inferior colliculus. Moreover, these data suggest that tottering and leaner mouse mutants are deficient in the signal which supresses TH expression in adult Purkinje cells. The identification of such a deficiency may aid in cloning and characterizing the tg gene. Supported by PHS CA33730, NS23038 and an American Epilepsy Society Research Fellowship.

383.8

MEANDER TAIL: GENETIC AND HISTOLOGICAL ANALYSIS OF A MOUSE MUTATION WHICH MAY DEFINE A DEVELOPMENTAL COMPARTMENT IN THE MAMMALIAN CEREBELLUM. Colin Fletcher*, Elizabeth Ross, Carol Mason, Mary Beth Hatten, and Nathaniel Heintz. Rockefeller University and Columbia University College of Physicians and Surgeons, NY, NY, 10021.

As part of a comprehensive program to identify the genes responsible for several aberrant neurological phenotypes in the mouse, we have adopted a molecular genetic approach to clone the gene product mutated in the meander tail mouse. Meander tail (*mea*) is a recessive mutation mapping to mouse chromosome 4, whose phenotype is characterized by both neurological and skeletal abnormalities. Thus, the adult *mea/mea* mouse displays normal foliation and cytoarchitecture in the posterior lobes of the cerebellar cortex. However, starting with an abrupt transition, Purkinje cells are completely disorganized in the anterior lobe: their dendrites extend in all directions, including into the white matter. Granule cells are sharply reduced in number at this transition and rapidly become indiscernible. Additionally, glial cell processes extend in all directions.

The *mea* locus has been fine mapped on chromosome 4 by analysis of several hundred progeny of an interspecific backcross to *Mus castaneus*. Closely linked markers are being used to isolate mouse YAC clones for the purpose of walking toward the locus.

The striking transition from normal to grossly disrupted cytoarchitecture involving at least three cell types in the *mea/mea* cerebellum suggests the presence of a discrete developmental compartment in the anterior cerebellum.

383.9

RESTING AND EVOKED RELEASE OF ENDOGENOUS DOPAMINE IN STRIATAL SLICES FROM THE WEAVER MUTANT MOUSE. Yu, H., Simon, J.R., Richter, J.A., Vasko, M.R. and Ghetti, B. Indiana Univ. Sch. of Medicine, Indianapolis, IN. 46202

Previous studies revealed that the weaver (wv) mutant mouse has a defect in the nigrostriatal dopaminergic pathway. The objective of this study was to determine if mice homozygous for the wv gene (wv/wv) exhibit an enhanced capacity to release striatal dopamine (DA) in order to compensate for the DA deficiency.

Striatal slices obtained from 3 month old wild type (+/+) and wv/wv mice were superfused and the release of endogenous DA was assayed by HPLC. Resting release of DA (expressed as a fraction of the tissue content) was significantly greater from wv/wv than from +/+ striatum (0.48%/min vs 0.24%/min). Fractional DA release evoked by 1 uM amphetamine was also significantly higher in the wv/wv (0.55%/min vs 0.29%/min), but there were no significant differences between +/+ and wv/wv in the fractional release evoked by either 24 mM or 48 mM potassium. These data suggest that under resting conditions, as well as during amphetamine stimulation, release mechanisms in the wv/wv striatum may adapt in an attempt to compensate functionally for the dopaminergic deficit. (Supported by RO1 NS 14426).

383.10

UPTAKE OF DOPAMINE, CHOLINE AND GABA IN THE STRIATUM OF WEAVER MUTANT MICE: TOPOGRAPHICAL DISTRIBUTION. J.R. Simon, A.B. Dunnington*, H. Yu and B. Ghetti. Indiana Univ. Sch. of Medicine, Indianapolis, IN. 46202.

The objective of the present study was to determine the topographical distribution of dopamine (DA), choline and GABA uptake in the striatum of control (+/+) and weaver (wv/wv) mice. Uptake of [3H]-DA, [3H]-choline and [14C]-GABA was determined in sucrose homogenates prepared from the dorsolateral (DL), dorsomedial (DM), ventrolateral (VL) and ventromedial (VM) portions of the striatum from +/+ and wv/wv mice. In 45-60 day old control mice, DA uptake was homogeneously distributed throughout the striatum. This distribution was altered in the wv/wv with the ventral aspects being less severely affected than the dorsal portions, though all areas exhibited significantly reduced uptake rates. In 9 and 12 month old mice choline uptake was higher in lateral than in medial aspects of the striatum of both genotypes with no differences being observed between genotypes. GABA uptake was evenly distributed in both genotypes, and again no differences were observed between +/+ and wv/wv mice. The present observations indicate that the entire weaver striatum is severely deficient in its ability to recapture DA and thus is functionally compromised. The results also indicate that striatal cholinergic and GABAergic interneurons are not directly or indirectly affected by the weaver gene. (Supported by RO1 NS 14426).

383.11

GENETIC PERTURBATION OF DENDRITE FORMATION. S. Roffler-Tarlov, A.M. Graybiel and B. Martin . Program in Neurosciences, Tufts Univ. Sch. Med., Boston, Ma 02111 & Dept. of Brain and Cognitive Sciences, MIT, Cambridge, MA 02139.

Weaver is an autosomal recessive mutation in mice that results in cell death among the dopamine-containing mesencephalic neurons and in cerebellum early in postnatal development. Previous observations made both of cerebellar granule cells and the dopamine-containing cells suggest that the gene affects neurite development. This hypothesis is supported also by the present comparison of tyrosine hydroxylase (TH)--stained cells in the midbrains of 7-day old mice of three genetic types: homozygous normal, heterozygous weaver and homozygous weaver. The examination was made before the onset of most of the cell death that takes place in the mesencephalon of the homozygous weaver. We saw a dramatic lack of TH-positive dendrites in homozygous weavers in areas of pars reticulata adjacent to densely packed TH-positive cell bodies. The weaver's midbrain contrasted markedly with that of the homozygous normal mice in which TH-positive dendrites formed a curtain of thickly aligned processes in the pars reticulata. In the heterozygous weaver in which dopamine-containing cells do not die, the pars reticulata was nevertheless only sparsely filled with dendrites.

The results show that the gene's effects on dendrites precede the death of vulnerable cells and that a single dose of the gene affects dendrite formation without causing cell death. Supported by NS20181 and MH00655.

383.12

TWO GENES FOR TEMPORAL LOBE EPILEPSY MAPPED IN THE MOUSE. M.L. Rise*, W.N. Frankel*, J.M. Coffin*, and T. N. Seyfried. Dept. of Biology, Boston College, Chestnut Hill, MA 02167, and Dept. of Molecular Biology, Tufts Univ. School of Medicine, Boston, MA 02111.

El is a neurological mutant strain of mice which is considered a model for complex partial seizures in humans (Seyfried and Glaser, Epilepsia 26: 143, 1985). Two Fl hybrid populations were generated from the following reciprocal crosses: El x ABP/Le and El x DBA/2J (D2). All of the Fl progeny from both crosses were seizure susceptible, substantiating previous evidence that El is inherited as a dominant trait. Beginning at 30 days of age, the mice were tested by vestibular stimulation twice a week for 10 weeks. The frequency of seizures per 20 tests was used to assess seizure susceptibility. The Fl mice were backcrossed (BC) to their respective non-El parental types. The offspring of both BCs were divided into Fl-like (seizure-susceptible) and D2- or ABP/Le-like (seizure-resistant) groups. Coinheritance of El with 6 conventional genetic markers and 46 endogenous proviruses was studied. El mice are wild-type at the se and d loci and express high ßgl activity, whereas D2 mice are d/d and ABP/Le mice are se/se, with both strains expressing low ßgl activity. A significant association was found between El and the chromosome 9 markers se and d, (which are tightly linked), and ßgl. The apparent recombination frequency between El and the d, se loci was 32%, and that between El and ßgl was 27%. Also, a significant negative association was found between El and two tightly linked chromosome 4 markers: b and an endogenous provirus. These findings indicate that El seizures result in large part from the actions of a major gene on distal chromosome 9, and a minor gene tightly linked to the b locus on chromosome 4. (Supported by NIH grant NS23355).

384.1

THE RELATIONSHIP OF INFARCT SIZE TO BLOOD PRESSURE AND TIME OF OCCLUSION DURING (PHOTOCHEMICALLY INDUCED) THROMBOTIC STROKE-IN-EVOLUTION. BD Watson, H Kanemitsu, R Prado, WD Dietrich, MD Ginsberg, Neurology, University of Miami, Miami, FL USA

The middle cerebral artery of halothane-anesthetized mature male Sprague-Dawley rats was exposed and irradiated with three independently positionable 457.9 nm laser beams. In order to lower the power threshold for thrombus formation and enhance lysis by t-PA, bixin (a singlet oxygen quencher) was injected with flavin mononucleotide (FMN) at 13.4-66 uM and 1.34-2.00 mM respectively. With two beams positioned distal to the rhinal branch and the other just below it, infarct area (A) at epicenter was proportional to the time of occlusion (t) in the blood pressure range of 90-110 mmHg for beams focussed sharply across the arterial diameter ($0<A<6.83$ mmHg^2, 40 min$<t<4$ hr, $n = 7$, $r = 0.96$). Diffuse beam focussing enhanced clot stability ($1.37<A<7.86$ mm^2, 3 hr$<t<4$ hr, $n = 5$, $r = 0.48$). At higher pressures (115 to 130 mmHg and diffuse focussing, A and t were not correlated. These results indicate that infarct susceptibility in thrombotic stroke is fundamentally conditioned by arterial blood pressure and collateralization. The rheological and histopathological characteristics of this minimally invasive and recanalizable (by endogenous or exogenous t-PA) model of arterial thrombotic stroke should provide insights into human clinical stroke.

384.2

ISCHEMIA INDUCES RELEASE OF GLUTAMATE IN REGIONS WHICH ARE SPARED FROM HISTOPATHOLOGICAL DAMAGE. M.Y.-T. Globus, R. Busto, E. Martinez*, I. Valdés*, and W.D. Dietrich. Cerebral Vascular Disease Research Center, University of Miami, School of Medicine, Miami, FL, 33101.

Excessive release of glutamate is thought to play a major role in the susceptibility of neurons to ischemia. In the present study we evaluated whether regional differences in the magnitude of glutamate release could explain selective vulnerability. Ischemia-induced changes in extracellular levels of glutamate in a region selectively vulnerable to 10 min of transient ischemia (CA1 sector of the hippocampus) were compared to the changes occurring in regions which, although rendered ischemic, are usually unaffected by a 10-min insult (thalamus, cortex and striatum). The degree of ischemia and the final histopathological outcome were also evaluated in these regions. Blood flow reduction and energy depletion were severe and uniform in all regions. The histopathological outcome illustrated a severely damaged CA1 sector of the hippocampus while all other brain regions were unaffected. Extracellular glutamate levels, measured by microdialysis, were significantly elevated during ischemia in all four regions. Glutamate levels continued to increase during the early recirculation period and gradually returned to baseline by 30 min of reperfusion, with similar temporal changes in all regions. These results demonstrate that elevated intra-ischemic glutamate levels of themselves are insufficient to engender ischemic damage, and other factors may play a pivotal part in the detrimental role of glutamate during ischemia *in vivo*.

384.3

POSTISCHEMIC (S)-EMOPAMIL THERAPY AMELIORATES FOCAL ISCHEMIC INJURY. E. Morikawa*, M.D. Ginsberg, W.D. Dietrich, and R. Busto. Cerebral Vascular Disease Research Center, Univ. of Miami, FL 33101.

In a previous study, (S)-emopamil (E), a calcium-channel blocker and serotonin S_2 antagonist, reduced cortical infarct volume in rat middle cerebral artery occlusion (MCA-O) (Nakayama et al, Neurology 38:1667, 1988). In this study, we explored the temporal window of therapeutic efficacy. Fifty-four fed male Sprague-Dawley rats underwent permanent proximal right MCA-O plus 30 min halothane-iduced hypotension to 50 mm Hg (Osborne et al, JNNP 50:402, 1987). There were 4 E-treatment groups: Group 1 (n = 9) received E 20-30 min prior to MCA-O; Groups 2 (n = 13), 3 (n = 12), and 4 (n = 8) received E 1, 2 and 3 hours, respectively, following MCA-O. Group 5 (n = 12) consisted of vehicle-injected controls. The initial E dose was 20 mg/kg IP with a 2nd dose 2.5 h later and twice daily x 2 days. Three days after MCA-O, brains were prepared for histologic quantitation of infarct area at 8 standard coronal levels; numeric integration yielded infarct volume. Cortical infarct volume in Groups 1-5, respectively, were (in mm^3, mean $\pm$ S.D.): Group 1, 84.5 $\pm$ 26.4; Group 2, 37.6 $\pm$ 27.6; Group 3, 47.8 $\pm$ 34.1; Group 4, 76.0 $\pm$ 26.9; Group 5, 72.9 $\pm$ 33.3. Striatal infarct volume was not affected. These data confirm a significant reduction of infarct volume with 1-h post-treatment and a trend at 2-h, suggesting a temporal window of efficacy.

384.4

MORPHOLOGICAL AND NEUROCHEMICAL SEQUELAE OF GLOBAL ISCHEMIA: COMPARISON OF SINGLE- AND MULTIPLE-INSULT PARADIGMS. B. Lin*, W.D. Dietrich, M.Y.-T. Globus, R. Busto, E. Martinez, S. Kraydieh and M.D. Ginsberg. Cerebral Vasc. Dis. Research Center, Miami, FL 33101.

To compare the consequences of global ischemia due to multiple vs. single insults, we subjected Wistar rats to either A) a single 15-min high-grade forebrain ischemic insult (n = 7), or B) three 5-min insults separated by one-hour intervals (n = 7). Ischemia was produced by bilateral carotid artery occlusion plus moderate hypotension (50 mm Hg) in rats whose cranial and rectal temperatures were separately thermostated at 36 and 37°C, respectively. Three days later, ischemic cell change (ICC) was quantitated. In hippocampus, caudoputamen, and cerebral neocortex, ICC was equally severe in Groups A and B. The ventrolateral thalamus, in contrast, showed moderate-to-severe ischemic injury in all rats of the multiple-insult group (B) but in only 1 of 7 rats in the single-insult group (A); numbers of ischemic neurons per high-power field were 4.7 $\pm$ 3.2 (mean $\pm$ S.E.) in Group A, and 30.8 $\pm$ 4.0 in Group B ($p < 0.01$). Preliminary intracerebral microdialysis data revealed 1) a delayed massive increase in extracellular striatal glutamate levels associated with 2) diminished extracellular glutamine 5-6 hours following the third multiple insult -- findings not observed in the single-insult group These results indicate cumulative effects of repeated normothermic insults.

Supported by Grants NS22603, NS05820, and NS26784.

384.5

EFFECT OF ISCHEMIA AND REPERFUSION IN VIVO ON ENERGY METABOLISM OF RAT SCIATIC-TIBIAL AND CAUDAL NERVES. P.J. Zollman*, O. Awad*, J.D. Schmelzer*, and P.A. Low. Neurophysiology Lab., Dept. Neurol., Mayo Fdn., Rochester, MN 55905

Our model of severe nerve ischemia consistently results in extinction of the compound nerve and muscle action potential (NAP; CMAP) within 30 minutes. Since impulse transmission may depend on nerve energy metabolism (NEM), we studied the effects of ischemia with reperfusion on NEM in vivo, in vitro and postmortem. Ischemia for 30 minutes postmortem or in deoxygenated Ringer's solution resulted in marked depletion of adenosine triphosphate (ATP), glucose (GLU) and creatine phosphate (CP) and an increase in lactate (LAC) of sciatic-tibial nerve of adult male Sprague-Dawley rats. In vivo ischemia for up to 3 hours in sciatic-tibial nerve was sufficient to extinguish CMAP but did not deplete ATP, CP, or GLU and did not increase LAC. Ischemia sufficient to extinguish NAP (caudal nerve) resulted in reduction of energy substrates to about 50% of resting. Muscle fails to conduct impulses before nerve and in vivo reductions of energy substrates are milder than in vitro changes.

384.6

EFFECT OF ISCHEMIA AND REPERFUSION ON OXYGEN FREE RADICALS IN RAT SCIATIC-TIBIAL NERVES. K.K. Ward*, J.D. Schmelzer*, N. Parinandi*, E. E. Benarroch, and P.A. Low. Neurophysiology Laboratory, Department of Neurology, Mayo Foundation, Rochester, MN 55905

Our model of severe nerve ischemia consistently results in extinction of the nerve impulse, reduced reflow and reperfusion injury to blood-nerve barrier. We therefore examined if oxygen free radicals (OFR) were generated during ischemia and reperfusion. Conjugated dienes (CD), hydroperoxides (OOH) and malondialdehyde (MDA) were measured in rat sciatic nerve. The endoneurial:epineurial ratios for CD, OOH and MDA were 3.46, 0.16 and 0.93 respectively, indicating that CD was mainly in endoneurium, OOH mainly in epineurium and MDA evenly distributed. In vitro deoxygenation resulted in no significant generation in OFR. Reoxygenation resulted in near doubling of MDA without changes in CD or OOH. Ischemia results in a reduction in OOH with a near 3-fold increase above the ischemic value on reperfusion. Smaller changes were seen with CD and MDA. These findings suggest that OFR is generated during reperfusion rather than during ischemia.

384.7

CEREBRAL ISCHEMIA AND REPERFUSION CAUSE BIPHASIC CHANGES IN EXTRACELLULAR DOPAMINE LEVELS IN THE STRIATUM. C.D. BLAHA, E.R. WOOD AND A.G. PHILLIPS. Dept. of Psychology, University of British Columbia, Vancouver, B.C., Canada.

Extracellular striatal dopamine concentrations were measured using 60s repetitive chronoamperometry in urethane anesthetised rats before, during and after transient forebrain ischemia. Stearate-modified carbon paste electrodes were implanted bilaterally into the striatum, and baseline extracellular dopamine was monitored for at least one hour. Ischemia was induced for 20 minutes by bilateral carotid occlusion combined with hemorrhagic hypotension to 30 mmHg. After this period, the carotids were unclamped and the shed blood reinfused over about 10 minutes. Extracellular dopamine levels followed a characteristic biphasic pattern: within three minutes of carotid clamping there was a 15-20 fold rise, followed by a gradual steady increase during the 20 minutes of occlusion. When the arterial clamps were removed there was an immediate decrease in the chronoamperometric signal. This was followed a few minutes later by a second 25-30 fold increase. The second rise in extracellular dopamine lasted for approximately 40 minutes, after which the concentration dropped gradually to a stable, elevated level with respect to the initial preischemic baseline. We suggest that the initial rise in extracellular dopamine is due to ischemic depolarisation of the neuronal membrane, whereas the second, larger increase is a result of reperfusion. Either of these increases in extracellular dopamine may be involved in striatal neuron death following cerebral ischemia.

384.8

EFFECTS OF DICHLORACETATE (DCA) ON LACTATE UPTAKE AND PYRUVATE DEHYDROGENASE ACTIVITY IN CULTURED ASTROCYTES. J.L. Tomsig[1], E. Gruenstein[2] and R.V.W. Dimlich[1,3]. Departments of [1]Emergency Medicine, [2]Molecular Genetics, Biochemistry and Microbiology and [3]Anatomy and Cell Biology. University of Cincinnati, Cincinnati, OH 45267.

Elevated brain lactate is an important contributor to the development of post-ischemic cerebral edema due to astrocytic swelling. DCA has been shown to reduce brain lactate in experimental cerebral ischemia in rats. Therefore we have investigated the effects of DCA on a human astrocytoma cell line, UC-11MG (Lomneth et. al., Brain Res.,486:95, 1989). Two hypotheses were tested: 1) DCA alleviates astrocytic swelling by increasing the rate of lactate metabolism via the stimulation of pyruvate dehydrogenase (PDHC) (E.C. 1.2.4.1) and 2) DCA decreases cell swelling by inhibiting the uptake of lactate into cells. We have found that exposure of cultured cells to 5 mM DCA for 30 min stimulates PDHC activity by 83%. We have also found that DCA acts as a competitive inhibitor of lactate uptake (Ki=1.7 mM at pH 6). Furthermore, cell swelling induced by exposure to 20 mM lactate at pH 6 for 30 min was blocked by 5mM DCA. These results are consistent with DCA acting to reduce ischemic injury by either or both of the proposed mechanisms. Supported in part by grants NIH BRSG SO7 RR 05408-28 and NS 25635 (RVWD), and NS 21663 and NS 27814 (EG).

384.9

GENDER-RELATED DIFFERENCES AND GLIAL CHANGES IN A RAT MODEL OF TRANSIENT FOREBRAIN ISCHEMIA. W.R.Woodward, H.Downes*, N.Lessov*, T.Williams* and C.K.Meshul. Depts. of Neurology, Biochemistry and Pharmacology, Oregon Health Sci. Univ. and VA Med. Ctr., Portland, OR 97201.

Transient forebrain ischemia (15 min) was produced in anesthetized ♂ and ♀ rats by bilateral carotid clamping coupled with reduction of blood pressure to 25 torr by rapid removal of blood. During ischemia the EEG became isoelectric, but recovered to normal levels once the carotids were unclamped and the blood reinfused. Animals were sacrificed at 7 d post-ischemia. In the post-ischemic period ♂ rats were slower to recover from the acute procedure than ♀ rats and lost rather than gained weight. In ♂ rats 57±3% of hippocampal circumference from CA1 through CA3 suffered severe neuronal damage (>75% loss) compared to 42±3% for ♀ rats. In both sexes neuron losses in CA1 were severe (≈85%) and less pronounced in CA3 (≈35%). Glia proliferated in CA1 (≈10-fold) and in CA3 (≈3-fold): rod-shaped microglia were prominent in stratum radiatum, and astrocytes and oligodendroglia increased in strata pyramidale and oriens. Gliosis occurred in animals even when there was little or no neuronal loss. We conclude that ♂ rats are more vulnerable to ischemia than ♀ rats and that glial proliferation can occur in areas where only relatively mild neuronal losses are observed. Supported by NIH grant NS17493 and the Veterans Administration.

384.10

ARE SODIUM CHANNELS OR SODIUM-CALCIUM EXCHANGE INVOLVED IN MEDIATING ANOXIC INJURY IN MAMMALIAN CENTRAL WHITE MATTER? P.K.Stys, B.R.Ransom, S.G.Waxman. Dept. of Neurology, Yale Univ., New Haven, CT 06510 and Neuroscience Research Center, VA Hospital, West Haven, CT.

White matter (WM) in the mammalian CNS suffers irreversible injury when exposed to extended periods (>30 min) of anoxia. Clinically, strokes involving WM can result in severe neurological disability. In spite of these facts, relatively little is known about the basic pathophysiology of WM anoxia. Using the isolated rat optic nerve (RON) as a model of central WM anoxia, we demonstrated the critical role played by extracellular Ca^{2+} (Stys, P.K. et al., Proc Natl Acad Sci USA, 1990 in press) and suggested that irreversible anoxic injury in WM depends on Ca^{2+} influx into a cytoplasmic compartment. We subsequently investigated how Ca^{2+} might enter cells during anoxia. Adult RONs were exposed to a standard 60 min period of anoxia. Functional recovery was assessed 1hr after re-oxygenation by measuring the area under the compound action potential. Blockade of voltage-dependent Na^+ channels with 1μM TTX significantly improved recovery (81.5±11.1% vs 38.3±10.6%, P<0.00001). This suggested that part of the damaging inward Ca^{2+} flux might occur through these channels. Alternatively, degradation of the transmembrane Na^+ gradient by channel-mediated Na^+ influx might cause reverse operation of the Na^+-Ca^{2+} exchanger, resulting in increased $[Ca^{2+}]_i$. This hypothesis was tested by blocking the exchanger with bepridil (10-100μM), a mechanism-based inhibitor of Na^+-Ca^{2+} exchange; this resulted in significantly enhanced recovery (59.5±13.4 % vs 38.3±10.6%, P<0.005). Inhibition of voltage-gated Ca^{2+} channels by bepridil is unlikely since dihydropyridines did not improve recovery (Stys, P.K. et al., Neurosci Lett, 1990 in press). Combined treatment with bepridil and TTX did not improve recovery (73.6±9.8%) over that seen with TTX alone. These results suggest that transmembrane Ca^{2+} influx during anoxia occurs, at least in part, as a result of reverse operation of the Na^+-Ca^{2+} exchanger. Thus TTX protects WM by slowing the rise in $[Na]_i$, which in turn affects the Na^+-Ca^{2+} exchanger; TTX may also block direct Ca^{2+} influx via Na^+ channels imperfectly selective for this ion.

384.11

TEMPORAL CHANGES DURING CEREBRAL EDEMA IN LACTATE AND PH MEASURED IN VIVO BY $^1H/^{31}P$-NMR. **S. Mun-Bryce, G. Rosenberg. Neurology Service, Veterans Medical Center, and the Dept. of Neurology and Physiology, Univ. of N.M., Albuquerque, NM 87131.**

Changes in brain metabolism during the development of brain edema were examined using in vivo ^1H- and ^{31}P-NMR with surface coils and depth pulses. We produced a collagenase-induced intracerebral hemorrhage in adult rats (Rosenberg et al., Stroke, 1990). Collagenase-injected animals had increased water content in the posterior brain region, and a high behavioral injury score. Fifty-five animals had two NMR recordings each prior to injury and at 4, 12, 24, 48, 36, 72 hrs after injury, in a GN300 7T, 89 mm vertical-bore spectrometer. ^1H spectra were graded for peak heights of lactate (1.3 ppm), N-acetyl aspartate (2.0 ppm), and PCr/Cr (3.0 ppm). Differences in chemical shift between Pi and PCr peak determined the pH values from ^{31}P spectra. Lactate was increased over pre-injury values at 4, 12, 24, 36, and 48 hrs ($p<0.05$), but corresponding pH was similar for all time points. These experiments indicate that an increase in brain lactate concentration may occur in the edematous region without a decrease in brain pH.

384.12

CI-977, A NOVEL KAPPA OPIOID RECEPTOR AGONIST, REDUCES INFARCT SIZE IN A MODEL OF FOCAL CEREBRAL ISCHEMIA. J.J. Cordon*, P.A. Boxer, M.A. Dominick*, F.W. Marcoux. Parke-Davis Pharm. Res. Div., Warner-Lambert Co., Ann Arbor, MI 48105

Both opioid agonists and antagonists have been shown to be beneficial in the treatment of ischemic injury. The current experiments determined the efficacy of CI-977 a novel, potent kappa-selective agonist (affinity=0.11 nM, mu/kappa ratio=905) in reducing damage resulting from focal ischemia. The left common carotid artery of F-344 rats was ligated along with coagulation of the middle cerebral artery at the rhinal fissure. CI-977 or vehicle (saline) was adminstered IV 30 min and 24 hr post occlusion. In one experiment animals were sacrificed 2 days post occlusion and the degree of damage assessed by exclusion of the dye TTC in 2 mm sections. CI-977 (0.05 to 0.5 mg/kg) produced dose-related reductions in infarct volume, which were significant at 0.1 and 0.5 mg/kg. In another experiment the animals were perfusion fixed with 10% buffered formalin at 10 days after occlusion and sections 6 μM thick were taken every 800 μ and stained with iron haematoxylin/cresyl-echt violet. CI-977, at 0.1 mg/kg significantly decreased the volume of ischemic damage by 57% as compared to saline control. At 0.5 mg/kg CI-977 produced a non-significant decrease of 27%. These data suggest that CI-977 may be beneficial in the treatment of abrupt occlusive stroke.

384.13

OPIOID RECEPTOR ANTAGONIST NALMEFENE STEREO-SPECIFICALLY INHIBITS GLUTAMATE RELEASE DURING GLOBAL CEREBRAL ISCHEMIA. S.H. Graham, H. Shimizu*, A. Newman,# P. Weinstein, and A.I. Faden. Dept. of Neurology and Neurosurgery, University of California, San Francisco, CA 94121 and Department of Applied Biochemistry, Walter Reed Army Institute of Research, Washington D.C. 20307#

The opioid antagonist nalmefene, which has increased affinity for κ-opiate receptors, attenuates the reduction in tissue glutamate after global cerebral ischemia/reperfusion (Faden et al, in review). In the present study, the effect of nalmefene upon excitatory amino acid release during global ischemia was studied using the technique of microdialysis. A 4 mm microdialysis probe was stereotactically placed in the dorsal hippocampus and perfused with mock CSF at 2 μL/min in 26 isoflurane anesthetized rats. One hour after insertion of the probe, complete global cerebral ischemia was induced for 30 minutes by the 7 vessel occlusion method. Saline, 0.1 mg/kg of (-) nalmefene or the inactive (+) enantiomer was given 15 minutes prior to ischemia. Samples of dialysate were collected every 10 minutes before, during, and after ischemia and amino acid content determined by HPLC. Peak dialysate glutamate during ischemia was significantly ($P<.05$) less than in (-) nalmefene treated animals (21.3±4.0 μM), while there was no difference between the saline (35.1±4.6 μM) and (+) nalmefene groups (34.9±5.0 μM). These results suggest that κ-opiate receptors may modulate glutamate release during ischemia and that inhibition of excitotoxin release may contribute to the protective actions of opioid antagonists in cerebral ischemia.

384.14

THE LACK OF EFFECT OF FLUNARIZINE ON PRESERVING NEUROLOGIC FUNCTION AFTER EXPERIMENTAL STROKE. W. M. Clark*, K. P. Madden, J. A. Zivin. Dept. of Neurosciences, UCSD, La Jolla, CA.

Prior studies using calcium entry blockers in experimental CNS ischemia have produced conflicting results. We studied the efficacy of Flunarizine (Janssen Research) in two models of selective CNS ischemia. A dose of 5mg/kg was given IV to treatment NZW rabbits 5 minutes post ischemia. In the first study, we used a model of spinal cord reperfusion to calculate the average length of ischemia required to produce paraplegia in each of the groups (ET_{50}). The ET_{50} in the Flunarizine group (N=17) was 29.8± 3.6 min., in controls (N=13) 24.1± 2.2 min.; P= .19. In the second study, we used microspheres to produce multiple brain infarcts. The average amount of microspheres required to produce abnormal neurologic function was calculated (ES_{50}). Agents which protect the CNS increase the ES_{50}. For the Flunarizine group (N=9), the ES_{50} was .36± .09 mg; in controls (N=14) .44± .12 mg; P= >.25. Thus, Flunarizine did not improve neurologic function in two sensitive models of CNS ischemia.

384.15

FPL 13950, AN ANTICONVULSANT WITH MARKED ANTI-ISCHEMIC PROPERTIES IN ANIMAL MODELS. G.C. Palmer, E.W. Harris, J. C. Strand*, M.L. Stagnitto*, A.R. Borrelli*, E.F. Cregan*, J.C. White*, R.J. Murray* and R.C. Griffith*. Fisons Pharmaceuticals, Div. R&D, Box 1710, Rochester, NY 14603.

FPL 13950 (formerly PR 1013-708 or 2-amino-N-[1,2-diphenylethyl]acetamide) is a moderately potent, safe, orally acting anticonvulsant which protects rodents against maximal electroshock seizures (ED50=20.5 in mice & 15.5 in rats; Garske et al., Soc. Neurosci. 14: 866, 1988). During routine screening with thermoregulated mice, oral FPL 13950 extended the time to death during hypoxia (ED50=19). Analogous tests in rats were confirmatory. In global ischemia models rats were subjected to 30 min. of 4-vessel occlusion, followed by 20 mg/kg FPL 13950 or saline i.p., immediately upon reflow and daily for 1 or 2 weeks. Drug-treated had much less CA1 cell loss and larger CA1 electrophysiological responses (orthodromic & antidromic population spikes recorded in vitro) than saline-treated rats. Dogs were subjected to 8 min global ischemia (clamping the ascending aorta), treating with FPL 13950 i.v. 30 min. post ischemia, then b.i.d. for 3 days and then once daily for 7 days). Histological examination confirmed that FPL 13950 markedly reduced ischemic damage in CA1.

These findings warrant continued development of this or similar compounds for eventual indications in patients suffering from cardiac arrest or coronary by-pass surgery.

384.16

REDUCTION IN ISCHEMIC INFARCTIONS PRODUCED BY OCCLUSION OF RAT MIDDLE CEREBRAL ARTERY BY TREATMENT WITH AGENTS ACTING AT IMIDAZOLE RECEPTORS. L. Pek*, K. Maiese, S.B. Berger and D.J. Reis. Div. of Neurobiol., Cornell Univ. Med. Coll., NY, NY 10021.

Treatment with the α_2-adrenergic (α_2) receptor antagonist idazoxan (IDA) reduces the size of the cerebral infarction produced by global cerebral ischemia (Gustafson, I. et al., *J. Cereb. Blood Flow Metab.* 9:171-174, 1989). It is not known whether: (a) IDA can modify a focal ischemic injury; (b) if its effect is entirely attributable to an interaction with α_2-receptors since IDA also binds to imidazole (IM) receptors. Thus, we compared the effects of IDA with the selective non-imidazole α_2-antagonist SKF 86466 (SKF) and the oxazole rilmenidine (RIL), an α_2-agonist which also binds at IM sites, on infarctions produced by occlusion of the middle cerebral artery (MCA) in rat. The MCA was occluded in rats anesthetized with isoflurane in 100% O_2 and drug immediately administered while maintaining arterial pressure and blood gases for 1h. Animals were sacrificed 24 h later, brains stained and lesions measured. IDA (3mg/kg) and RIL (1mg/kg) both significantly ($p<0.05$, n=5 each group) reduced lesion size compared with controls (IDA, 153±4.5 mm^3; RIL, 139±7.8 mm^3; control, 196±6.6 mm^3; n=5). SKF (15 mg/kg) had no effect (186±6.5 mm^3; n=5). We conclude: (a) IDA and RIL can reduce a focal ischemic infarction; (b) the effect of IDA cannot be attributed to antagonism of α_2-receptors. An interaction with IM receptors may underlie the tissue preserving actions of IDA and RIL.

384.17

CORTICOSTERONE EXACERBATES ISCHEMIC-LIKE INJURY IN MIXED HIPPOCAMPAL CULTURES. G.C. Tombaugh and R.M. Sapolsky. Dept. of Biol. Sci., Stanford Univ., Stanford, CA 94305

Glucocorticoids can potentiate ischemic neurotoxicity in the rat hippocampus. However, the cellular and biochemical mechanisms underlying this synergy are not known. To begin to characterize this effect we have used primary hippocampal cultures, containing both neurons and astrocytes (~1:1), derived from 18d fetal rats. At 10-12 days of age, cultures were refed with experimental medium containing 5mM glucose with or without 100nM steroid. 24hr later, cultures were refed with steroid-free medium containing varying glucose concentrations, immediately made hypoxic (100% N_2) for 6hr, then refed with fresh medium (5mM glucose). The following day, cell damage was measured by lactate dehydrogenase assay. Pretreatment with corticosterone (CORT) enhanced hypoxic cell damage under low (0.4mM) or no-glucose conditions but had no effect when glucose = 5mM. In normoxic controls, CORT was damaging only in the "no-glucose" group even though glucose-free exposure alone caused no significant damage. Neither progesterone, estrogen, nor testosterone had any measurable effect. Thus, CORT aggravates hypoxic injury in hippocampal cultures only under conditions that are likely to cause energy failure and subsequent cell death. These results are consistent with the idea that glucocorticoids enhance cell vulnerability by weakening cellular energy state. In support of this model, glucocorticoids have been shown to inhibit glucose transport into cultured hippocampal neurons and astrocytes.

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384.18

HYPERTENSIVE RATS PRODUCE MORE TUMOR NECROSIS FACTOR THAN NORMOTENSIVE RATS IN RESPONSE TO CHALLENGE WITH LIPOPOLYSACCHARIDE. J.M. Hallenbeck*, D.A. Doron, [1]G. Feuerstein, [2]H.B. Pollard and E. Heldman. Dept. of Neurology, U.S.U.H.S., Bethesda, MD 20814 and [1]Dept. of Pharmacology, Smith, Kline and French laboratories, King of Prussia, PA 19406 and [2]Lab. Cell Biol. and Genetics, NIH, Bethesda, MD 20892.

Hypertension is one of the most common risk factors for stroke. Spontaneous hypertensive rats (SHR) have been demonstrated to experience a high incidence of ischemic and hemorrhagic lesions in the brain stem, following a provocative dose of lipopolysaccharide (LPS). LPS stimulates macrophages to secrete the cytokines interleukin-1 (IL-1) and tumor necrosis factor-α (TNF-α), both of which convert the endothelium surface from an anticoagulant to a procoagulant state and thus may cause the production of focal ischemic lesions. In order to determine if increased TNF-α activity were one of the factors which may be responsible for the high incidence of ischemic lesions in LPS-treated rats, we challenged SHR and normotensive rats (WKY) with 1.8 mg/kg LPS and measured TNF-α levels in the blood and cerebral spinal fluid (CSF). We found that LPS induced a marked elevation of TNF-α activity in both SHR and WKY. However, the response of the SHR was significantly higher than that of the WKY controls, resulting in a significantly higher TNF-α level in the blood of SHR 2 h following challenge with LPS. Furthermore, when LPS was delivered intravenously, TNF-α activity was elevated mainly in the blood but not in the CSF. By contrast, when LPS was injected intracerebroventricularly, TNF-α was high in the CSF and low in the blood. These results strongly suggest that TNF-α is produced locally in the brain and thus may cause focal ischemic lesions. The higher incidence of stroke-like events occurring in SHR as compared to WKY may be related to the higher level of TNF-α obtained after challenge with LPS.

384.19

ACETAZOLAMIDE-OUABAIN INHIBITS CEREBROSPINAL FLUID PRESSURE RISE BY CEREBROVENTRICULAR INFUSIONS IN RATS.

Morrow, BA*, LC Keil and WB Severs, Hershey Medical Center, Penn. State Univ. School of Medicine, Hershey, PA 17033

Cerebrospinal fluid pressure (CSF-p) rises 2-3 fold about 2 hr after an 8 μl/min x 10 min infusion of artificial cerebrospinal fluid (aCSF) in conscious rats. (*FASEB J.*, 4:A1095, 1990) Elevated resistance to outflow (Ro) accompanied the elevated CSF-p. Acetazolamide (ACTZ), a carbonic anhydrase inhibitor, and ouabain, an inhibitor of Na^+/K^+ ATPase, were both employed to prevent the formation of CSF. Six Male S-D rats (300-400g) were surgically implanted with a sealed 4 mm cerebroventricle cannula (1.5 mm l & 1.0 mm p to bregma) and allowed to heal for 2 days. The evening prior to the experiment, ACTZ, 50 mg/kg, s.c., was given. On the test day, the rats received both ACTZ, 50 mg/kg, i.p., and ouabain, 2.5 μg/5 μl aCSF, ivt, just before connecting to the recording system. Ouabain caused a brief 1-2 min seizure after a 1-3 min delay. Thirty min after dosing, the rats received the infusion, 8 μl aCSF/min x 10 min. Ro and compliance (Comp) were evaluated by boluses of aCSF (10 μl/0.5 sec) at 20 min and 4 hr after the infusion. CSF-p's were not different ($p>0.05$). However, Ro rose (*=$p<0.05$), while Comp fell (*=$p<0.05$). In this study, the increase in CSF-p, but not Ro, was prevented by blocking CSF formation. These data indicate that increased CSF-p is not essential for increased Ro. We propose that elevated Ro causes the increase in CSF-p.

	20 min	4 hr
CSF-p, ($cmH_2O \pm$ SEM)	10 ± 2	13 ± 3
Ro, ($cmH_2O/\mu l/min \pm$ SEM)	1.5 ± 0.6	7.8 ± 2.1 *
Comp, ($\mu l/cmH_2O \pm$ SEM)	2.1 ± 0.6	0.6 ± 0.1 *

CSF-p's of rats receiving the 8 μl/min aCSF infusion with no drug therapy were 9.3 ± 0.9 and 23.6 ± 2.8 ($cmH_2O \pm$ SEM) for 20 min and 4 hr, respectively.

384.20

Physiological and Metabolic Changes During Gravity (+Gz) Induced Loss of Consciousness (G-LOC) in Rat Brain. A. R. Shahed, F. G. Aldape*, J. A. Barber* and P. M. Werchan*. USAF School of Aerospace Medicine, Op. Tech. Corp., and Krug Int., Brooks AFB, San Antonio, TX 78235.

High sustained or rapid onset of +Gz is known to cause G-LOC in pilots of high performance aircraft with potentially grave consequences. Relatively little is known about G-LOC and so approaches to its prevention are limited. In the present study a small animal centrifuge (SAC) was used to investigate the neurophysiological mechanism of G-LOC. Rats with surgically implanted electrodes for EEG, ECG and heart rate were loaded on a SAC equipped with a freeze blowing device. Control rats received identical surgical treatment but were not centrifuged. Rats were centrifuged for 30s at 1 to 32.5 Gz to determine G-tolerance and time for G-LOC to occur. Brains were frozen immediately (group 1), or 30-60s (group 2) after G exposures and analyzed for energy metabolites. At 25 + 1 Gz (n=19), G-LOC was observed within 17 + 1s and EEG remained isoelectric for 15-24s. Brain glucose decreased (70%) in group 1 and remained unchanged in group 2 over controls. Brain lactate increased 2- 2.5 fold over control in both groups. ATP and creatine phosphate levels decreased and AMP, ADP and adenosine levels increased significantly in both groups. This is the first report to show metabolic changes during G-LOC. Investigations continue to determine if these and/or other changes contribute to G-LOC.

384.21

^{31}P-NMR STUDIES OF ISCHEMIA IN NG108-15 CELLS: EFFECTS OF PHENYTOIN AND MG^{2+} ON RECOVERY OF HIGH ENERGY PHOSPHATES. F.A.X. Schanne*, P.K. Stanton, B.H. Smith and J.R. Moskal. Dept. Neurosurgery, Albert Einstein Col. Med, Bronx, NY 10467.

Intracellular high energy phosphates (HEP) were monitored in differentiated cells by ^{31}P-NMR under conditions of continuous perfusion, no flow and reperfusion to model changes occuring during cerebral ischemia and reperfusion. NG108-15 cells were attached to Cytodex microcarrier beads and differentiated for 5 days in dbcAMP (1 mM). Medium was supplimented with creatine (10 mM) 24 hrs before NMR observation. Cells on beads were transferred to an NMR tube and perfused with artificial cerebrospinal fluid at a flow rate of 1 ml/min. Cells were maintained at 37°C and the perfusion medium was saturated with 95% O_2/5% CO_2 (pH 7.4). Under these perfusion conditions, stable intracellular HEP resonance signals were observed using a Varian VXR 500 spectrometer with a 10 mm probe. Upon stopping the perfusion, there were rapid decreases in pH and in phosphocreatine (PCr), followed by losses in nucleotide phosphates. Upon reperfusion, the pH returned to normal and the HEP recovered towards normal levels, depending upon the duration of no flow. These changes follow a pattern similar to that observed by ^{31}P-NMR of cerebral ischemia *in vivo*. Following 40 min of ischemia and 40 min of reperfusion PCr returned to 52+/-4% of preischemia levels. Treatment with phenytoin at 5ug/ml and 20 ug/ml enhanced recovery to 75+/-6% and 73+/-7%, respectively. 10 mM Mg improved recovery to 80%. These data demonstrate that phenytoin as well as Mg are protective against neuronal damage induced by ischemia. Thus this model system should prove useful in helping to elucidate some of the molecular mechanisms underlying the neurotoxicity induced by ischemia.

ISCHEMIA V

385.1

FOREBRAIN ISCHEMIA INDUCES SELECTIVE BEHAVIORAL IMPAIRMENTS ASSOCIATED WITH HIPPOCAMPAL CA1 INJURY. J.D. Thomas, T. X. Gionet*, C.R. Goodlett, D. S. Warner, M. M. Todd*, E.A. Wasserman*, and J.R. West. Dept. of Psychology, Anesthesiology, & Anatomy, Univ. of Iowa, Iowa City, IA 52242.

Studies of forebrain ischemia in rats have demonstrated significant loss of hippocampal CA1 neurons and deficits in working memory. The selectivity of the behavioral deficits induced by forebrain ischemia was evaluated using six tasks, tested sequentially: retention of a radial maze discrimination [postoperative days (PO) 5-16], open field activity (PO 18), general motor performance (PO 18), step-through passive avoidance (PO 20-21), the Morris place learning task (PO 26-27), and rotarod performance (PO 28). All rats were trained on a 12-arm radial maze discrimination (6 arms baited and 6 arms unbaited) for 50 days, one trial per day. They were then randomly assigned to either the ischemia condition (n=8) or to the sham condition (n=6). Ten minutes of ischemia with an isoelectric EEG was produced by bilateral carotid artery occlusion and systemic hypotension (mean arterial pressure=50±5 mm Hg). Controls underwent the same surgery except ischemia was not induced. In the postoperative radial maze test, the ischemic group committed significantly ($p<.05$) more working memory errors than controls during the first block of six trials, but recovered to control levels by the second block. The ischemic group also had a nonsignificant trend toward more reference memory errors in the first block. The groups did not differ significantly in open field activity, motor performance, passive avoidance performance, spatial learning in the Morris navigation task, or balance on the rotarod. Neuronal loss in CA1 was rated (scored 0 to 3) from 5-µm coronal sections by an investigator blind to group membership and behavioral performance. The severity of working memory deficits was associated with the extent of CA1 damage. Furthermore, if only the rats with severe, bilateral CA1 loss were considered (n=4), passive avoidance retention deficits were also evident. In summary, none of the tests of motor function distinguished the groups, indicating that generalized motor dysfunction is not a reliable index of the outcome of forebrain ischemia in this rat model. However, ischemia resulted in relatively specific cognitive impairments that were associated with the extent of hippocampal CA1 neuronal loss. (Supported by grants #GM 39771 to DSW and #AA 05523 to JRW)

385.2

Immunohistochemical study of "Delayed Neuronal Death" with anti–NF200 antibodies. Y. Kaku, Y. Yonekawa, N. Ogata and T. Tsukahara. Department of Neurosurgery, National Cardiovascular Center, Suita, Osaka 565, Japan.

Selective vulnerability and "delayed neuronal death" of hippocampal CA1 region have been well documented, but its precise mechanism has not been clarified so far. Our previous report demonstrated that the degradation of the neurofilament (NF) triplet proteins occurs in cerebral ischemia and suggested that it may be related to irreversible neuronal cell death (Ogata et al. J. Neurosurg,70:103,1989). In this study, "delayed neuronal death" was induced in a rat by lowering the systemic systolic blood pressure to 50 mmHg in addition to temporal occlusion of the bilateral common carotid arteries, followed by reperfusion. The duration of the ischemia was 2 min, 5 min, and 8 min; and the duration of reperfusion was 1 day and 7 days. The histology was confirmed by hematoxylin–eosin (HE) staining and by immunohistochemical staining with anti–NF200 antibodies (NF staining). In 8 minutes ischmia, HE staning showed the survival of most of the neurons in the CA1 region at 1 day after ischmia and more than 75 % loss of the neurons in the same region at 7 days after ischemia. In contrast, NF staining showed that the reactivity to anti–NF200 in the CA1 region was already decreased at 1 day and remained to be low at 7 days after ischemia. Immunoreactivity in the CA3 region, in which almost all the neurons survived, was unchanged both at 1 day and at 7 days after ischemia. These results suggest that the decrease of the immunoreactivity to anti–NF200 precedes the neuronal cell death.

385.3

RESPONSE OF SOMATOSTATIN NEURONS TO TRANSIENT FOREBRAIN ISCHEMIA IN THE GERBIL: AN IN SITU HYBRIDIZATION STUDY. J. L. Seeburger, J. E. Springer, and C.-S. Lin. Depts of Neurology and Physiology & Biophysics, Hahnemann University , Philadelphia, PA 19102-1192.

The selective vulnerability of specific populations of forebrain neurons to ischemic insult has been well documented. These include neurons in the hippocampal CA1 and dentate hilar areas, layers 2/3 and 6 of the somatosensory neocortex, the dorsolateral striatum, and the lateral septum. Recently, somatostatin positive neurons have been found in these regions. We therefore investigated the response of these somatostatin neurons to ischemic insult. Because different forms of the somatostatin molecule are derived from a single precursor and individual neurons may not express all forms, an in situ hybridization procedure was used to localize neurons containing somatostatin mRNA. Ischemic lesions were produced by 5-min bilateral occlusions of the common carotid arteries in Mongolian gerbils. Fresh sections were collected on a cryostat after 2d, 4d, and 2wk post-occlusion survival periods. Sections were processed with a nonradioactive oligonucleotide probe complementary to somatostatin mRNA. Adjacent sections were stained with Cresyl Violet or silver. A decrease in somatostatin mRNA signal was noted in regions known to be sensitive to ischemic damage as early as 2d post-occlusion. Similar results were obtained with the 4d and and 2wk survival conditions. Furthermore, cell loss and argyrophilia were noted in the same regions stained with Cresyl Violet and silver, respectively. Therefore, the loss of somatostatin mRNA signal was most likely due to cell death. It remains possible, however, that surviving cells ceased to express somatostatin mRNA. Our present results suggest a correspondence between the localization of somatostatin neurons and neurons sensitive to ischemic damage in the gerbil forebrain. Supported by NIH S07RR07241.

385.4

EFFECT OF TRANSIENT FOREBRAIN ISCHEMIA ON SOLUBLE PROTEINS FROM CA1 AND CA3 REGIONS OF THE HIPPOCAMPUS. E. A. Heinicke* and A. M. Buchan, Robarts Research Institute, London, Canada.

CA1 pyramidal neurons of the hippocampus die 2 to 3 days after transient forebrain ischemia, whilst neurons of the CA3-dentate gyrus region survive. The distribution of soluble proteins from these regions was examined for changes that might lead to cell death.

Male Wistar rats were subjected to 15 min. transient forebrain ischemia (4-VO model), followed by reperfusion for 1, 3 or 7 days. Normal, untreated rats served as controls. Soluble proteins from CA1 and CA3-dentate regions were separated electrophoretically by SDS-PAGE, stained with Coomassie Blue, and those of less than 87 KD (constituting 83% of the applied sample) were quantitated by densitometer.

The banding patterns from the two regions were identical, with two exceptions; two bands, 58 and 11 KD, occurred in different proportion in CA1 and CA3 in all groups of animals. Only three protein bands - 76, 54 and 26 KD - were affected quantitatively by ischemia. The constancy of the composition of the cytosolic protein compartment of the two regions before and after ischemia suggests that protein breakdown may not be a major factor in ischemic neuronal death.

385.5

DISTRIBUTION OF 70 KDa HEAT SHOCK PROTEIN mRNA INDUCTION AFTER TRANSIENT GLOBAL ISCHEMIA IN THE RAT. T. S. Nowak, Jr., G. Nagashima*, K. Kawai* and I. Klatzo. Laboratory of Neuropathology and Neuroanatomical Sciences, NINDS, NIH, Bethesda, MD 20892.

Recent studies have documented the induction of the 70 kDa stress / heat shock protein, hsp70, following transient ischemia in the gerbil, and have suggested its utility as an early marker for neuronal circuitry which may participate in the evolution of subsequent pathology. Since postischemic protein synthesis deficits may variably limit the capacity of cells to express immunoreactive hsp70 protein, localization of hsp70 mRNA by in situ hybridization has proved a more reliable index of the stress response. This method was applied in the present study to evaluate the distribution of hsp70 mRNA in rat brain after 10 min transient global ischemia subsequent to cardiac arrest produced by compression of the major thoracic vessels, followed by resuscitation. Results obtained at 6 h recovery, the time of maximal hsp70 induction in the gerbil ischemia model, demonstrate a generally similar distribution of hybridization in the rat, including all hippocampal pyramidal neurons as well as the dentate granule cell layer, lateral striatum, piriform cortex and neocortex. In contrast to the gerbil, modest hybridization was also apparent in ventral thalamic nuclei, which subsequently show evidence of cellular injury, as well as serum protein and calcium accumulation, in this model. These results provide further support for the use of hsp70 hybridization in the molecular mapping of postischemic neuropathology.

385.6

EXPRESSION OF HEAT SHOCK PROTEIN 70 mRNA FOLLOWING REVERSIBLE FOCAL ISCHEMIA IN RAT BRAIN F.A.Welsh and D.J.Moyer. Div. of Neurosurgery, Univ. of Pennsylvania, Philadelphia, PA 19104.

To investigate the relationship between the expression of heat shock proteins and ischemic brain damage, in situ hybridization was performed in postischemic rat brain using an oligonucleotide probe specific for inducible 70-Kd heat shock proteins (HSP-70). Reversible focal ischemia was produced by transient occlusion (1 hr) of both carotid arteries combined with permanent occlusion of the middle cerebral artery. After reperfusion for 2 hr, intense expression of HSP-70 mRNA was evident in the area of ipsilateral neocortex known to undergo infarction. However, HSP-70 mRNA was also expressed in regions not normally injured, including paramedian neocortex, striatum, and hippocampus. After 24 hr reperfusion, expression of HSP-70 mRNA was still evident in neocortex and CA_1 subfield of hippocampus, but not in striatum. These results demonstrate that the postischemic expression of HSP-70 mRNA occurs both in regions undergoing ischemic injury and in regions that recover without histologic evidence of damage.

385.7

METABOLIC VIABILITY AND PROTON HOMEOSTASIS IN THE HIPPOCAMPAL SLICE: EFFECT OF ACID LOADING. H.A. Assaf*, T.S. Whittingham, J.C. LaManna, R.A. Ratcheson, and W.D. Lust. Lab. of Experimental Neurological Surgery, Case Western Reserve University, Cleveland, OH 44106.

Intracellular acidification secondary to lactic acid accumulation has been implicated in the evolution of brain damage following an ischemic episode. It has been shown that lactic acid in an acidic medium is more toxic than HCl to cells of neural origin grown in culture (Goldman et al., J. Cereb. Blood Flow Metab., 1989), suggesting that uncharged organic acids may facilitate the flux of protons across membranes. Similar experiments were performed on hippocampal slices to determine the effect of a number of weak acids on pHi during an acid load. The metabolic viability (i.e., ATP levels) and pHi, derived from the creatine kinase equilibrium, were determined in hippocampal slices incubated for up to 30 min in media acidified to pH 4.9 with the following acids: hydrochloric (H), pyruvic (P), D-lactic (DL), L-lactic (LL), formic (F) or acetic (A) acids. While ATP levels in the H and P groups were maintained for up to 30 min (> 80% of cont), those in the remaining groups were depleted (<25% of cont) by 30 min. The loss of ATP in the F and A groups was complete within 5 min of incubation, whereas ATP depletion occurred between 10 and 30 min in the DL and LL groups. The pHi at 10 min of incubation dropped from a control value of 7.62 $\pm$ 0.04 to 7.41 $\pm$ 0.06, 7.20 $\pm$ 0.08, 7.12 $\pm$ 0.20* and 6.61 $\pm$ 0.07* for the H, P, LL and DL groups, respectively (*, $p < 0.05$). In contrast, the pHi decrease in the A and F groups was greater and at 5 min was 6.29 $\pm$ 0.15* and 5.60 $\pm$ 0.21*, respectively. The observed differences in hippocampal slice pHi appear to be determined by both the pK' and lipid solubility of the acids. The ability of weak organic acids to enhance the movement of protons suggests that this process may modulate localized acid-base imbalances following anoxia/ischemia.

385.8

DIALYSIS PERFUSION EXACERBATES STRIATAL ISCHEMIC DAMAGE. L.A. Phebus+, R.E. Mincy* and J.A. Clemens. The Lilly Research Laboratories, Eli Lilly and Co. +and The Program in Medical Neurobiology, Indiana Univ., Indpls. In 46285

Ten minutes of four-vessel occlusion (4-VO) in the rat usually produces no striatal ischemic damage. We tested the effect of dialysis probe implantation and perfusion on the striatal cell loss produced by 10 min of 4-VO.

Male Wistar rats were prepared for 4-VO by permanently cauterizing the vertebral arteries and placing an atraumatic snare loosely around the carotids. At the same time, a "loop type" microdialysis probe was stereotaxically inserted in the anterior, lateral striatum. The next day, the rats were placed in a circular chamber and the dialysis probe was perfused at a rate of 1 uL/min with an artificial CSF solution containing 150 mM NaCl, 3 mM KCl, 1.7 mM $CaCl_2$ and 0.9 mM $MgCl_2$ with a pH of 7.4. After about 2 hours of perfusion, 4-VO was produced in the animals by closing the snares around the carotids. Loss of righting reflex was used as an index of adequate ischemia. After 10 min. the snares were opened and reperfusion begun. At least 24 hours later, the rats were sacrificed and striatal damage was assessed histologically. In eleven rats, there was never any ischemic cell loss in the non-perfused striatum. In the perfused striatum, 10 of the 11 rats showed some cell loss and 5 of these showed severe cell loss symmetrically distributed around the dialysis membrane.

385.9

REDUCED PARTICULATE PKC ACTIVITY DURING FOCAL CEREBRAL ISCHEMIA. R.C. Crumrine, W.R. Selman*, J.C. LaManna, K.A. Seta*, W.D. Lust. Department of Neurology and Division of Neurosurgery, University Hospitals of Cleveland, Cleveland, Ohio 44106.

Protein kinase C (PKC) is a component of intracellular signaling systems. Activation of PKC has been implicated in the modulation of neuronal excitability, neurotransmitter release and modulation of intracellular pH. Thus, altered PKC activity may have an influence on the ability of cerebral tissue to survive an ischemic insult. Occlusion of the left middle cerebral artery (MCA) in the spontaneously hypertensive rat strain results in a consistent and reproducible area of tissue damage. The MCA was occluded for 6 hours with a snare ligature and then the rats were frozen in situ. The brains were removed and sectioned in a microtome (-20^{0}C) at the level where the right and left aspects of the corpus collosum fused. Representative histological sections confirmed a focal ischemic event and were used for dissecting the appropriate regions. Tissue samples for PKC assay were then obtained from the dorso-lateral cerebral cortex both ipsilateral and contralateral to the occlusion. PKC was assayed by measuring the incorporation of ^{32}P from the gamma position of ATP into exogenous histone (type IIIs).

Total PKC activity (particulate + soluble) in the ipsilateral cerebral cortex was depressed by 56% as compared to the contralateral cerebral cortex (1.24±0.44 vs 2.84±0.14 nmol/min/mg protein; NS, paired t-test). The proportion of particulate PKC activity in the ipsilateral cerebral cortex was 52% of that of the contralateral cerebral cortex (17.6±0.6% vs 33.8±2.0%; p<0.05, paired t-test). Thus, the detectable particulate PKC activity was only 25% of normal in the ipsilateral cerebral cortex after 6 hours of focal ischemia. The marked reduction in activity of this second messenger system may not only influence tissue survival during stroke, but also may retard functional recovery following reperfusion.

385.10

SEGMENTAL BLOOD FLOW IN ISCHEMIC SPINAL CORD OF RABBITS. K.E. Peek, J. Goddard-Finegold, M.J. Joerger*, C.S. Robertson*. Dept. of Neurosurgery, Baylor College of Medicine, Houston, TX 77030.

Blood flow before, during, and/or after spinal cord ischemia may affect neurological recovery. Eight New Zealand albino rabbits, anesthetized with ketamine, were artificially ventilated with supplemental oxygen. A balloon catheter was positioned in the abdominal aorta just distal to the origins of the renal arteries. Somatosensory evoked potentials were recorded at L5-L6 during sciatic nerve stimulation, and functional spinal cord ischemia was determined by the disappearance of postsynaptic potentials following balloon inflation. Segmental spinal cord blood flow (sSCBF) was measured in the 9 caudal segments using 15-micron radiolabeled microspheres at 4 times: before and during ischemia and at 30 and 120 min reperfusion.

Before ischemia, mean±SD sSCBF varied from 12.2±3 mL/100g/min at T12-L1 to 25.7±10 mL/100g/min at L7-S1. During ischemia, sSCBF was reduced between 31.7%±33 (L1-L2) and 91.3%±12 (L7-S1). At 30 min reperfusion, hyperemia was evident at all segments, with the greatest percent change from pre-ischemia values (179%±74) at L7-S1. By 120 min reperfusion, sSCBF values had normalized to control levels at all segments. sSCBF may prove to be an important correlate in acute and chronic measures of functional recovery from spinal cord ischemia.

385.11

TISSUE OXYGENATION AND EVOKED POTENTIAL RECOVERY AFTER GLOBAL CEREBRAL ISCHEMIA. Z.-C. Feng, T.J. Sick and M. Rosenthal. Dept Neurology, Univ Miami Med Sch, Miami, FL 33101
. Recovery following cerebral ischemia may be impaired by effects of reperfusion and 'oxidative stress.' Recordings by optical and electrode techniques in control rats anesthetized with pentobarbital and respired with 30% O_2 showed that reperfusion after ischemia (30 min; 4-vessel occlusion model) is accompanied by: a) recovery of extracellular potassium ion activity (K^+o); b) hyperemia; c) tissue hyperoxygenation; and d) hyperoxidation of cytochrome a,a_3. However, clearance of K^+ after its elevation by direct cortical stimulation remained slowed. Present studies examined effects of increasing or decreasing the fraction of oxygen in the inspired gas mixture (FiO_2) one minute prior to reperfusion. This was done to manipulate amplitudes and durations of post-ischemic mitochondrial hyperoxidation (PIMHo) and tissue hyperoxygenation which were increased by high FiO_2 and decreased or suppressed by low FiO_2. In the former animals, K^+o baseline remained elevated (approx 1 mM) above baseline and there was significantly poorer recovery of somatosensory evoked potentials and EEG activity. In animals respired with 15% O_2 during the first 10 minutes after reperfusion, recovery of evoked potential and EEG activity was enhanced over control animals. Clearance of K^+o following stimulation was not significantly influenced by changes in FiO_2. We suggest that: a) hyperoxygenation and PIMHo residual metabolic dysfunction after ischemia; b) the events may be related to reperfusion or 'oxidative stress' injury; c) events associated with PIMHo and tissue hyperoxygenation may decrease electrophysiological recovery; and d) suppression of PIMHo and tissue hyperoxygenation by respiration of a slightly hypoxic gas mixture in the early post-ischemic period may have beneficial effect.

385.12

SELECTIVE CORTICAL ISCHEMIA AFTER OCCLUSION OF A MIDDLE CEREBRAL ARTERY IN GERBILS. O. Takemoto* and T. Yanagihara. Dept. of Neurology, Mayo Clinic, Rochester, MN 55905

We developed an experimental model of selective cortical ischemia by occlusion of a middle cerebral artery through the suprazygomatic retro-orbital approach in gerbils and followed the evolution of ischemic damage from 30 min to 7 days by using the immunohistochemical reactions for α-tubulin and microtubule associated protein (MAP) 1 and 2. Five gerbils were used for each ischemic period. Ischemic lesions were detected first in the frontoparietal cortex in the layer III/IV at 30 min, in all gerbils after 1 hr with the reaction for MAPs and after 2 hrs with the reaction for α-tubulin. Extensive lesions developed in 12 hrs. Ischemic lesions in the parietal cortex progressed more slowly. Laminar lesions were visible with the reaction for MAPs at 3 hrs, but only a few scattered lesions were recognizable with the reaction for α-tubulin. It took 24 hrs before all gerbils developed extensive lesions in the parietal cortex. This model may be useful for a comparative study of the ischemic focus and the surrounding area with marginal blood flow in the cerebral cortex.

385.13

EFFECTS OF BILATERAL CAROTID ARTERY OCCLUSION (BCO) ON $GABA_A$ RECEPTOR FUNCTION IN MONGOLIAN GERBIL BRAIN Beth E. Mileson and Rochelle D. Schwartz, Dept. of Pharmacology, Duke University Medical Center, Durham, NC 27710.

The selective neuronal damage which occurs in the hippocampus (HIP), striatum (STR) and cortex (CTX) following transient forebrain ischemia is thought to be mediated by excessive neuronal stimulation. We examined the possibility that a loss of inhibitory neurotransmission may compound the overstimulation and contribute to cell death. $GABA_A$ receptor function in occluded and sham-operated mongolian gerbils was measured in synaptoneurosomal preparations of HIP, STR and CTX.

Gerbils were allowed to recover from a 5 minute BCO for 1, 4 or 29 days. Since the degree of HIP, STR and CTX damage following 5 minute BCO may vary, all gerbils were screened one day after occlusion for locomotor activity in a novel environment. An increase in this measure is a reliable indicator of HIP damage in the gerbil. A "mini" dose-response curve of muscimol-stimulated ^{36}Cl uptake was produced for each brain region using 2, 5, and 20 μM muscimol. No difference in GABA gated Cl^- uptake was seen in any brain region 1 or 4 days after BCO compared to controls, though a loss of binding to the Cl^- channel of $GABA_A$ receptors has been observed in BCO gerbils on day 4 (see next poster). Twenty-nine days after BCO, no changes in Cl^- uptake were seen in the STR or CTX, but a 30 % decrease was seen in the HIP of BCO gerbils.

These results do not support the hypothesis that a loss of $GABA_A$ receptor function plays a role in overstimulation and neuronal death, but they do not rule it out. GABA neurotransmission may be impared in specific populations of neurons within a brain region, but not detected in synaptoneurosomes from an entire brain structure. Supported by NIH AG00029 (BEM); NS24577, AHA grant in aid (RDS); RDS is an Established Investigator of the American Heart Association

385.14

EFFECTS OF BILATERAL CAROTID ARTERY OCCLUSION (BCO) ON THE $GABA_A$ RECEPTOR/CHLORIDE CHANNEL IN MONGOLIAN GERBIL BRAIN: AUTORADIOGRAPHY USING ^{35}S-TBPS. Martha L. Ehrmann*, Beth E. Mileson, Patricia P. Edgar, and Rochelle D. Schwartz, Dept. of Pharmacology, Duke University Medical Center, Durham, NC 27710.

The role of GABAergic neurotransmission following transient forebrain ischemia was investigated in brain regions selectively vulnerable to neuronal injury. Gerbils were subjected to 5 min of BCO and allowed to recover for 1 or 4 days. Behavioral and histologic analyses were performed to assess the extent of CA1 hippocampal damage. A significant correlation was observed between the extent of CA1 hippocampal degeneration and increased locomotor activity. Gerbils with > 100% increase in locomotor activity were subsequently used for autoradiography. Slices from the cortex, striatum, hippocampus, and cerebellum were incubated with 1 nM ^{35}S-TBPS and exposed to film for autoradiography (Edgar and Schwartz, J. Neurosci. 10:603-612, 1990). Significant decreases in TBPS binding 4 days following BCO were observed in the hippocampus: stratum oriens (CA1, 35%; CA2, 20%), CA1 molecular layer (44%), stratum radiatum (CA1, 14%; CA3, 14%), and in the lateral striatum (55%), n=9. No changes in binding were observed in the cortex or cerebellum. These studies indicate that the binding of a probe to the Cl^- channel of the $GABA_A$ receptor is significantly altered in certain regions of the gerbil hippocampus and striatum that were damaged following transient ischemia. This may represent altered Cl^- ion channel activity associated with loss of specific neurons. Our ongoing studies will indicate whether receptor binding changes occur prior to neuronal degeneration and/or if compensatory changes occur over time following neuronal degeneration.

Supported by the PMA Foundation, NIH NS 24577, and AHA Grant in Aid (RDS). RDS is an Established Investigator of the American Heart Association.

385.15

THROMBOSIS IN PARIETAL CORTEX OF RATS IMPAIRS LEARNING IN A 14-UNIT T-MAZE. D. Ingram, E. Spangler*, B. Jones, P. Garofalo*, M. Jucker, J. Long, M. Pontecorvo. Gerontol. Res. Ctr., NIA, NIH, Baltimore, MD 21224; Nova Pharmaceutical Corp., Baltimore, MD 21224.

Using a photochemical method (Watson B. *et al., Ann Neurol* 17:497, 1985), focal thrombosis was produced in parietal cortex (PCtx) of 3-mo old male F-344 rats. One wk after implantation of jugular catheters, experimental rats (n=7) were anesthetized by halothane; given scalp retraction; injected with rose bengal dye (60mg/kg) via catheter; and then illuminated on cranium (bregma coordinates: A/P=-1.6;M/L=4.3 mm) with a high intensity light source for 40 min bilaterally. Controls (n=7) received identical treatment except for dye injection and skull illumination. After 10 days rats were pretrained in 1-way active avoidance to criterion (8 avoidances/10 trials), and 24 hr later received 15 trials in a 14-unit T-maze with performance sensitive to aging and septo-hippocampal damage (Ingram D., *Neurobiol. Aging*, 9:475, 1988) as well as to aspiration lesions of PCtx during acquisition (Berman R. *et al. Soc. Neurosci. Abst.*, 14:234, 1988) but not retention (Jucker M. *et al. Physiol. Behav.* 47:207, 1990). Rats locomoted to goal through 5 maze segments each within 10 sec to avoid footshock. Compared to controls in the T-maze, PCtx-damaged rats had increased errors, runtimes, shock frequencies and durations. In pretraining, no differences were observed in shock avoidance. Thus, the infarct primarily affected cognitive performance in the maze. Histological assessment showed infarct usually confined to PCtx with no hippocampal encroachment but occasional occipital cortex damage. Additional experiments using occipital cortex infarct will determine the significance of this damage. However, results suggest PCtx involvement in the age-related learning deficit in this task and provide a model of cerebral ischemia with a well-defined functional impairment.

385.16

PHOTOCHEMICALLY-INDUCED ISCHEMIA OF THE FRONTAL CORTEX RESULTS IN A TRANSIENT MEMORY IMPAIRMENT IN RATS. D. B. Clissold, B. E. Jones, R. G. Cutler*, and M. J. Pontecorvo. CNS Pharmacology, NOVA Pharmaceutical Corp., Baltimore, MD 21224.

Exposure of the rat motor cortex to an intense light in the presence of a fluroescin dye initiates a sequence of events, including local thrombosis and subsequent infarct, that mimic the events occurring during a stroke in man. We now report that it is possible to produce a bilateral infarct in the dorsal-medial prefrontal cortex of the rat which results in a reliable impairment of delayed alternation memory performance.

Rats were trained to alternate responses between two levers. Intertrial (retention) intervals of 2.5, 5 and 10 seconds were randomly distributed within each session. Upon reaching criterion rats were assigned to a Lesion or Sham group (n=5). The lesion was produced by administering the fluroescin dye rose bengal, i.v., exposing the skull, and centering a 6 mm diameter beam of high intensity light 2 mm anterior to Bregma for 60 minutes. The lesion produced a significant, retention interval-dependent impairment of alternation accuracy over the first post-operative week . Mean percent correct at the 2.5, 5 and 10 sec intervals was 97, 89, and 78 in the Sham and 96, 87, and 69 in the Lesion group. With continued testing, the magnitude of the deficit diminished. The lesion did not affect one measure of performance (probability of any response) but slightly increased the latency to a response (reaction time).

385.17

THE EFFECTS OF TRANSIENT ISCHEMIA ON THE M1 MUSCARINIC RECEPTOR IN GERBIL FOREBRAIN. G.R. Luthin and C.S. Lin. Department of Physiology and Biophysics and Institute for Neuroscience, Hahnemann University, Philadelphia, PA 19102.

Transient ischemia produces selective neuronal death in gerbil forebrain. The goal of this study was to establish the pattern of staining of another marker, the m1 subtype of muscarinic acetylcholine receptor (m1 mAChR), following ischemia. This marker was chosen based on our recent observation that regions known to contain high levels of m1 mAChR are those most affected following ischemia. An anti-peptide antiserum was produced in rabbits, affinity purified and used to localize the m1 mAChR in gerbil brain. Ischemia was produced by bilateral carotid occlusion (5 minutes). 5-7 days following the occlusion, gerbils were sacrificed, perfused with 3% paraformaldehyde, and brains were sectioned with a vibratome. Tissue was blocked with 10% normal donkey serum, incubated with primary and secondary antibodies, and the m1 mAChRs were localized using DAB as substrate for HRP. The brain regions showing a decrease of m1 mAChR staining directly paralleled those evidenced by staining with silver. These include the hippocampus, but not the dentate, and lateral portions of the caudate-putamen. These data indicate that there is a direct correspondence between the neuronal areas containing a high concentration of m1 mAChRs and those most vulnerable to transient ischemia.
(Supported by NS 23006 and NIH S07RR07241)

385.18

SILICONE CYLINDER EMBOLIZATION AS A RAT STROKE MODEL. A.D. Perez-Trepichio and S.C. Jones. Cerebrovascular Res. Lab., Cleveland Clinic Foundation, Cleveland, OH 44195

Reliable stroke models are important for further investigation of the pathophysiology and treatment of stroke. Silicone cylinder embolization, in contrast to the middle cerebral artery (MCA) occlusion, avoids craniotomy, leading to a less traumatic model.

Seven male Sprague-Dawley rats weighing (293 ± 6 g, mean ± SEM) were anesthetized with pentobarbital (50 mg/kg ip). Body temperature was maintained at 37°C during the surgery. Unipolar EEG was recorded over the MCA region. The left common carotid (LCC) was exposed and the pterygopalatine artery electrocoagulated. The LCC was temporarily clamped, for less than 10 min to allow cannulation of the left external carotid. In five animals (embolized) a silicone cylinder (900 μm long, 300 μm diameter) was then infused into the left internal carotid with normal saline. Two sham animals received only normal saline. EEGs were obtained before and after embolization, under anesthesia and 24 hr later in the unanesthetized state and were analyzed using Fourier frequency analysis. Total surgery time was 80 min. After 24 hr the animals were decapitated and 2 mm brain slices were obtained. The slices were incubated with 2,3,5-triphenyltetrazolium chloride in phosphate buffer at 40°C for 30 min and fixed in formalin. The infarct volume was determined using digitized image analysis for at least 8 photographed sections for each animal.

The infarct volume ratio, expressed as percentage of total brain, was 10.68 ± 1.86%, n = 5 in embolized animals (mean ± SEM) and 0.15 ± 0.15%, n = 2 for the sham animals (mean ± range). The EEG analysis showed an immediate decrease in amplitude following embolization in the ipsilateral hemisphere. One hour and 24 hours after embolization, clinical observation showed persistent rotation to the right. This model of focal cerebral ischemia mimics, closely a clinical embolic stroke and could be developed into an awake stroke model.

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385.19

DEVELOPMENT OF A NO-REFLOW MODEL OF CEREBRAL ISCHEMIA IN THE RAT USING ANGIOGRAPHY. **R.M. Kline,* T. Panetta,* B.V. Updyke and N.G. Bazan, LSU Eye Center and Neuroscience Center, New Orleans, LA 70112**

The no-reflow phenomenon and its role in the pathophysiology of human stroke are poorly understood. No-reflow is seen in rat models of global cerebral ischemia incorporating cerebrospinal fluid compression or artificially induced hypotension, although neither of these is a feature of human stroke. Because the production of no-reflow is related to the severity of ischemia produced, models that produce no-reflow may be used to help distinguish neurochemical events of pure ischemia from those due to reperfusion. Thus, a more physiologic model of no-reflow would provide unique opportunities to compare ischemic-phase events to reperfusion-phase events.

A novel *in-vivo* angiographic technique using intravascular $BaSO_4$ has been employed to identify collateral sources of blood flow in pure ligation models of cerebral ischemia. Systematic surgical elimination of observed collateral flow was achieved by permanent ligation of the internal thoracic and vertebral arteries at their origins, followed by temporary ligation of the subclavian and common carotid arteries. The hydrogen clearance technique was used to measure average cortical blood flows of 2.7 ± 1.3 cc/100g/min and midbrain flows of 3.3 ± 1.3 cc/100g/min during ischemia. No-reflow was observed in 73% of electrodes upon attempted reperfusion. Use of a standardized neurologic scoring system after 20 min of ischemia and 1 h of reperfusion documented a consistently severe clinical insult. This model provides the unique opportunity to study the effects of severe global ischemia without the additional variables of artificially increased intracranial pressure or artificially induced systemic hypotension.

385.20

DISTRIBUTION OF 14C-PHENYTOIN IN RAT BRAIN FOLLOWING MIDDLE CEREBRAL ARTERY OCCLUSION (MCAO). W.P.McNally, P.DeHart*, J.J.Cordon, D.M.Rock, C.P.Taylor. Parke-Davis Pharm. Res., Ann Arbor, MI 48105

In several rodent models phenytoin (P) has been reported to reduce neuronal cell loss following temporary global ischemia or permanent focal ischemia resulting from MCAO. We examined the distribution of P in brains of MCAO (with ipsilateral carotid artery occlusion) and sham operated rats. Animals were given 30 mg/kg(50 µCi) 14C-phenytoin IV, 30 min. after surgery and sacrificed under halothane anesthesia at 0.25, 1, 2, or 4 hrs after dose for sectioning and quantitative autoradiographic analysis. Radioactivity (R) was evenly distributed in gray matter at 15 min. although slightly lower levels were seen in neocortex on the operated side of MCAO brains. Only a relatively small (2-4mm) area originating in agranular-parietal cortex contained significantly lower R than surrounding tissue, in spite of the fact that in separate studies of matched rats kept two days after MCAO much larger areas were infarcted. From 1 through 4 hours R in this small area (core of infarct) increased and was considerably higher than surrounding cortex or sham operated cortex at 4 hr. Distribution patterns suggest diffusion of R into ischemic regions presumably from residual collateral circulation. Thus, P distributes homogeneously and rapidly to brain tissues despite greatly reduced blood flow in MCAO induced focal ischemia in the rat. Persistence of R in core of infarct may result from lack of circulation in the area.

386.1

SEX DIFFERENCE IN VULNERABILITY TO SEVERE FOCAL CEREBRAL ISCHEMIA. K.E. Pazara*, K.L. Linseman*, P.A. Yonkers and E.D. Hall. CNS Dis. Res., The Upjohn Co., Kalamazoo, MI 49001.

Possible gender-related differences in ischemic and post-ischemic pathophysiology and neuronal damage were examined in Mongolian gerbils subjected to a 3 hr. period of unilateral carotid occlusion (UCO). Only gerbils showing neurological signs of UCO-induced cerebral ischemia were studied. In initial experiments, it was observed that females displayed significantly less 24 hr. post-ischemic neuronal necrosis in both the hippocampal CA_1 and lateral cortical regions. Males exhibited 34.8% more necrosis in CA_1 and 38.3% more in the lateral cortex. In a second set of mechanistic experiments, no difference was observed in cortical blood flow between the two sexes before, during or for 2 hr. after the 3 hr. UCO. Moreover, the ischemic decline in cortical extracellular calcium did not differ, but the post-ischemic recovery was significantly greater in the females. Measurement of brain vitamin E levels as an index of lipid peroxidation (LP) showed a 43.5% decline by 2 hrs. after reperfusion in males while in females the decline was only 4.2% ($p<0.05$). Previous studies showing protective effects of antioxidants in this model have suggested an important role of oxygen radical-induced LP. Thus, it is postulated that the lesser ischemic vulnerability and preservation of brain vitamin E in females may be due to an antioxidant effect of endogenous estradiol.

386.2

COUPLING OF METABOLISM AND ELECTRICAL ACTIVITY IN CORTICAL ASTROCYTES IN CULTURE. D.E. Harold and W. Walz. Dept. of Physiology, Univ. of Saskatchewan, Saskatoon, Sask., S7N 0W0, Canada.

The effect of metabolic inhibitors and of ouabain on membrane potential and input resistance of primary cultures of astrocytes from newborn Swiss mice were evaluated. Astrocytes always react to metabolic inhibitors with an immediate depolarization and not with hyper-polarizations as neurons. Antimycin A caused depolarizations of up to 50 mV. These depolarizations are always reversible even after a 60 min exposure time. Thus astrocytes are well capable of surviving a prolonged hypoxic insult. The glycolytic inhibitors Na fluoride and iodoacetic acid caused an initial depolarization rate of 0.6 respective 0.7 mV/min, which is less than the 1.2 mV/min caused by ouabain. Addition of ouabain did not exceed the 1.2 mV/min rate. Thus it seems that inhibition of glycolysis exerts its effects by Na^+, K^+ pump blockade via ATP depletion. Long-term exposure (>20 min) to the reversible glycolytic inhibitor Na fluoride caused irreversible effects depending on the amount of depolarization which were accompanied by a 3-5 times increase in resistance. Astrocytes were usually capable of recovering well from similar depolarizations caused by ouabain. Thus, we conclude that inhibition of glycolysis is causing irreversible damage, which is not directly related to the resulting ion gradient breakdown.

386.3

MEASUREMENT OF HYDROXYL RADICALS BY QUANTITATION OF METHANE SULFINIC ACID (MSA). M.A. Elchisak, L. Kendall,* C. Babbs,* and M.C. Scott. Dept Vet Physiol and Pharmacol, Purdue Univ, West Lafayette, IN 47907.

Production of hydroxyl free radicals is difficult to measure due to their short half-lives. We report here a method to measure MSA, the immediate and stable product of a trapping reaction between a hydroxyl radical and DMSO. We used HPLC with dual-coulometric electrode electrochemical detection. The column was silica-based trimethyl (Burdick and Jackson, 5 micron) and the mobile phase (pH 3.4) was sodium acetate (100 mM) and tetrabutylammonium hydroxide (8 mM). Output was monitored from the second working electrode (W2). MSA produces a response approximately 30,000 times greater than DMSO, thus allowing measurement of small amounts of MSA in the presence of large amounts of DMSO, even though the resolution (alpha) between the two compounds is only 1.2. The detector response was linear for MSA between 0.39 and 200 pmol. This method was utilized to quantitate MSA in perfusate from the transendothelially perfused rat lung to study pulmonary generation of hydroxyl radicals during reperfusion injury. No sample preparation was required prior to the HPLC analyses. The within-assay coefficient of variation (CV) for these samples ranged from 0.40 to 1.44 percent (n=3); the between-assay CV was 5.4% (n=2).

386.4

BRAIN DAMAGE IN THE NEWBORN PIGLET AFTER HYPOXIA AND SEVERE PNEUMOTHORAX. C.S. Easley*, A.E. Kopelman*, F.S. Wartman*, T. Conner-Kerr*, and T.M. Louis. Depts. of Pediatrics and Anatomy/Cell Biology, East Carolina University School of Medicine, Greenville, NC 27858.

We examined the effect of severe pneumothorax (SP) in combination with hypoxia on brain histopathology in 32 newborn piglets. This model parallels hypoxic ischemia seen in newborn infants with air block syndrome. We randomly assigned the piglets to 4 groups: control, hypoxia only, SP only, or a combination of hypoxia and SP. We produced hypoxia in halothane-anesthetized piglets by having them breathe a 1:1 mixture of air and nitrous oxide for 2 hours. We then induced SP by injecting air into the right pleural cavity until the mean systemic blood pressure fell to 33% of baseline and was maintained for 4-16 minutes. Blood pressure, heart rate, blood gases, and blood chemistries were monitored throughout the surgical procedures. We resolved the SP and re-stabilized and maintained the piglets for 3-7 days. The basal ganglia, neocortex, and hippocampus were examined for pathological changes using a silver impregnation method highly selective for degenerating neurons. The brain regions were scored by an independent observer for the presence or absence of damage and analyzed using a Fisher's exact test. Damage was found only in the animals exposed to a combination of SP and hypoxia. Neurons containing silver precipitate were found in the dorsal lateral basal ganglion and the depths of neocortical sulci. We rarely found degenerating neurons in the hippocampus. (Partially supported by the NC Affiliate of the American Lung Association).

386.5

EFFECTS OF HYPOXIC ISCHEMIA ON SOMATOSENSORY EVOKED POTENTIALS IN NEWBORN PIGLETS. T.A. Conner-Kerr*, T.M. Louis, R.H.Ray. Depts. of Anatomy & Cell Biology and Physiology, East Carolina Univ., Greenville, NC 27858

The purpose of this study was to determine the effects of hypoxic ischemia on the somatosensory evoked potential (SEP) of the newborn piglet. Newborn piglets under halothane anesthesia received one of the following treatments: sham (n=3), hypoxia (n=3), ischemia (n=2), hypoxic ischemia (n=4). SEPs were recorded at the following times: baseline, 1 hour, 2 hours, 3 hours, and 7 days after treatment. SEPs were also recorded during the 4 minute ischemia, 8 minutes, 15 minutes, and 30 minutes after ischemia. Stimuli were delivered to the volar pad of the forelimb (20 mv intensity, 0.3 ms duration, 1/s). The active recording electrode was placed intradermally over vertex and the reference electrode was clipped to the ear. A Nicolet 1170 was used to average the SEPs over 128 stimulus trials. SEPs of animals which received hypoxia alone did not become isoelectric at any time. However, SEPs were isoelectric during ischemia in both the ischemia only and hypoxic ischemic animals. Waveshape analysis indicated that the waveshape remained changed after 7 days only in animals which received ischemia and hypoxic ischemia. We conclude that SEPs provide a reliable noninvasive technique for early detection of events leading to hypoxic ischemic encephalopathy in newborns.

386.6

PERINATAL HYPOXIC-ISCHEMIC (HI) BRAIN INJURY ALTERS STRIATAL AMINO ACID EFFLUX IN RAT BRAIN: AN IN VIVO MICRODIALYSIS STUDY. K Gordon, J Simpson, D Statman, FS Silverstein. Depts of Pediatrics and Neurology, University of Michigan, Ann Arbor, MI.

We used microdialysis (MD) to determine the influence of an acute HI insult on striatal extracellular fluid (ECF) excitatory amino acid (EAA) efflux in perinatal brain. In 7 d.o. rats, MD probes were inserted into the right striatum. To induce HI injury, the right carotid artery was ligated and animals were exposed to 8 % O2 for 2.5 hr (n=22). Ischemia alone (n=10), hypoxia alone (n=8), and untreated controls (n=17) were also studied. With an HPLC-EC assay, glutamate (GLU), aspartate, asparagine, serine, glutamine, glycine, taurine (TAU), & alanine were consistently detected in dialysates; baseline GLU efflux was 2 pmol/min. In untreated controls, efflux values were stable over 4 hr. During HI, efflux values fluctuated widely and there was marked intra- and inter-animal variability. Significant changes in HI animals included: transient increases in GLU (peaks > mean+2 SD) in 0/17 controls & 8/22 HI, $p<0.03$, 2-tailed, Fisher's exact test; increased taurine efflux in 10/22 HI (vs 1/17 controls), $p = 0.01$, and in 7/8 HI animals with the greatest increases in GLU ($p<.006$). ASP efflux did not change during HI. GLU and TAU efflux did not change in ischemia- or hypoxia- alone controls. In this experimental model of perinatal HI brain injury 1) HI stimulates transient bursts of GLU release, 2) TAU efflux rises co-incident with GLU accumulation, and 3) patterns of striatal EAA efflux differ considerably from findings in stroke models in mature brain.

386.7

AGE AND SUBSTRATE DEPENDENCE OF ANOXIC DAMAGE IN RAT HIPPOCAMPAL SLICES. E.L. Roberts, Jr. and T.J. Sick. Department of Neurology, University of Miami School of Medicine, Miami, FL 33136

Metabolic substrate concentrations and type were altered to see if the diminished recovery of ion transport and of synaptic transmission following anoxia in hippocampal slices from aged rats (26-27 mon.) (Roberts, E.L., Jr. *et al.*, *Brain Res.*, in press, 1990) might be due to age-related changes in glycolysis or oxidative phosphorylation. Hippocampal slices from rats of ages 6 (young adult) and 26 (aged) mon. were superfused with an artificial cerebrospinal fluid containing 0-20 mM glucose, and subjected to anoxia (95% N_2, 5% CO_2). Normoxia (95% O_2, 5% CO_2) followed anoxia one minute after complete loss of ion homeostasis (anoxic depolarization) in a slice. In 0 mM glucose, 20-30 mM sodium lactate was added to the superfusate to support oxidative phosphorylation. As glucose concentration increased, the duration of anoxia needed for anoxic depolarization was prolonged in young adult slices. After anoxia, the recovery of ion transport and synaptic transmission, as assessed respectively from reuptake of K^+ and from the orthodromic population spike recorded in hippocampal subfield CA1, improved as glucose concentration increased, but recovery was less in aged slices. Slices exposed to sodium lactate maintained K^+ homeostasis prior to anoxia, but varied greatly in their retention of synaptic excitability. Recovery of synaptic transmission following anoxia in sodium lactate solution was usually not seen. The faster loss of ion homeostasis during anoxia in aged slices may be due to a defect in glycolysis. Also, glycolysis may be necessary for recovery of synaptic transmission following anoxia. (Work supported in part by a research grant from the American Society for Aging Research).

386.8

NEUROPROTECTIVE EFFECTS OF ADENOSINE AGONISTS ON CEREBRAL ISCHEMIA IN THE GERBIL. R.L. Dean, D. Lutz, S. Mennerick and R.T. Bartus. CORTEX Pharmaceuticals, Inc., Irvine, CA 92718

Bilateral occlusion of the carotid arteries in the gerbil (a model of cerebral ischemia in humans), followed by reperfusion, results in elevated glutamate levels which lead to hippocampal degeneration. Adenosine A1 receptors exist in areas particularly affected by ischemia (e.g., hippocampus, striatum, cerebral cortex). This distribution is similar to that of the glutamate receptor subtype, NMDA. Since adenosine analogues are potent inhibitors of the presynaptic release of glutamate and down-modulate the postsynaptic effects of NMDA stimulation, adenosine agonists may be useful for the treatment of ischemia and stroke.

This study was designed to determine: 1) the effect of adenosine as a neuroprotectant when compared with NMDA receptor system antagonists, and 2) the neuroprotective role of A1 vs. A2 adenosine receptor-specific compounds. First, we directly compared the effects of pre-occlusion administration (ip) of the adenosine analogue, ADAC, with the selective NMDA antagonist, CPP, and the non-competitive NMDA antagonist, MK-801, for their ability to protect both histologically and behaviorally against Ischemia-induced CA1 neurodegeneration (5 minute occlusion). Next, the effects of a selective A1 agonist (CHA), A2 agonist (DMPA), and the mixed A1/A2 adenosine agonist (NECA) were directly compared to further characterize the neuroprotectant role of adenosine in this model. Because adenosine agonists produce overt side effects (e.g., sedation, hypothermia), the peripherally vs. centrally mediated protective actions were also characterized and will be discussed.

386.9

ADENOSINE ANTAGONISTS FAIL TO PREVENT ANOXIC G_K-MEDIATED HYPERPOLARIZATION OF HIPPOCAMPAL NEURONS. K. Krnjević and Y. Xu. Anaesthesia Research Dept., McGill University, Montréal, PQ., Canada H3G 1Y6.

Adenosine (ADN) has a G-protein mediated hyperpolarizing effect on hippocampal neurons (Segal (1982): Eur. J. Pharm. 79, 193). Anoxia causes a liberation of ADN from brain tissue (Berne et al. (1974): Circ. Res. 35, 262). If ADN is responsible for the hyperpolarization seen early during anoxia in pyramidal neurons (Hansen et al. (1982): Acta. Physiol. Scand. 115, 301), its action should be susceptible to block by ADN antagonists. In CA1 pyramidal cells of rat hippocampal slices at 34°, brief anoxia (2-3 min) usually evoked a reversible hyperpolarization, increase in conductance, and loss of action potentials. In 9 slices, superfusion with caffeine (in doses ranging from 0.05 to 1.0 mM) tended to augment synaptic responses and caused some progressive depolarization; but the main anoxic effects (hyperpolarization, conductance increase and inexcitability) were not consistently reduced. Similar negative results were obtained in 7 other slices when applying the even more specific ADN antagonist 8(p-sulfophenyl)theophylline. We conclude that the anoxic hyperpolarization of hippocampal pyramidal cells cannot be principally caused by adenosine release. (Supported by MRC of Canada).

386.10

ATP DOES NOT CORRELATE WITH RECOVERY FROM ANOXIA WITH THIOPENTAL IN THE RAT HIPPOCAMPAL SLICE. **I.S. Kass, J.E. Cottrell* and G. Chambers*. Anesthesiology Dept. State University of New York, Health Science Center, Brooklyn, NY 11203**

We used the in vitro hippocampus as a model system to examine whether CA 1 pyramidal cells are protected against anoxic damage by thiopental. The postsynaptic evoked population spike was recorded from the CA 1 pyramidal cells after stimulation of the Schaeffer collaterals. Significance was determined using ANOVA and t-tests ($p < .05$); values are mean ± standard error.

When the CA 1 pyramidal cells are subjected to 3.5 min. of anoxia the postanoxic population spike recovers to only 10±4 % of its preanoxic amplitude. If slices are treated with thiopental (600 uM) 15 min before,during and 10 min after anoxia there is 67±10 % recovery of the response. Thus thiopental significantly improved recovery of the response after anoxia. A lower concentration of thiopental (250 uM) also showed significant protection (24±2 %), however there was a clear dose related effect. The lowest dose of thiopental tested (100 uM) did not show significant protection (17±6 %).

Thiopental (600 uM) significantly decreased the level that ATP fell to in the CA 1 region during 3.5 min of anoxia (2.3±.11 nM/mg dry wt., untreated; vs. 1.6±.07,thiopental). After 5 min of anoxia there was no significant difference between treated and untreated slices. Only after 10 min of anoxia did thiopental show a slight preservation of ATP during anoxia.

Thus neurons in the rat hippocampal slice recover better from short periods of anoxia if they are treated with high concentrations of thiopental but this recovery does not correlate with ATP levels during anoxia.

386.11

BEHAVIORAL CHANGES FOLLOWING VARIOUS PERIODS OF TEMPORARY ISCHEMIA IN MONGOLIAN GERBILS. E. Fadda, S. Mazzari, M.M. Zanellato*, R. Zanoni*, A. Zanotti*, G. Toffano and A. Leon. Fidia Research Laboratories, 35031 Abano Terme, Italy

In gerbils, transitory forebrain ischemia can be easily produced by bilateral occlusion of common carotid arteries (CCAo) and there occurs, following reperfusion, delayed neuronal death in the CA_1 hippocampal region. We studied the changes of locomotor activity consequent to different periods of CCAo (1, 2, 3 and 5 min) at 1, 2, 6, 24, 96 hours after reperfusion. CCAo was performed in overnight fasted female gerbils (50-60 gr of body weight) after their exposure under local anesthesia: mortality rate was lower than 5%. Histopathological changes were examined in coronal sections stained with cresyl violet and silver impregnation. Locomotor activity significantly increased only following 3 and 5 min ischemia, reaching in both cases a maximum at 6-24 hrs after reperfusion and then declining. Degeneration of CA_1 pyramidal cells was evident only after 3 min of CCAo and after 5 min of ischemia, neuronal death was observed also in somatosensory cortex. These data indicate that in gerbils 1) the evolvement of hippocampal neuronal death is associated with augmented locomotor activity and 2) the behavioral and histologic changes are dependent on the duration of CCAo.

386.12

FOCAL CEREBRAL ISCHEMIA: CHARACTERIZATION OF INFARCT EVOLUTION AND EFFECTS OF GM1 TREATMENT. M.S. Seren, T. Koga*, N. Schiavo*, A. Lazzaro*, G. Vantini, G. Toffano and A. Leon. Fidia Research Laboratories, 35031 Abano Terme, Italy

In the experimental model of rat middle cerebral artery occlusion (MCAo) according to Tamura, both cortex (mainly the parietal portion) and striatum are severely damaged. In these two areas we evaluated the temporal profile of infarct progression by assessment of water accumulation (edema), ionic alterations as well as loss of characteristic neurotransmitter-related parameters. Cerebral edema appeared already at 3 hrs after MCAo reaching its maximum at 24-72 hrs and thereafter declining. Calcium and sodium accumulation showed a similar trend. Furthermore, focal ischemia induced a severe and long-lasting loss of choline acetyl transferase (ChAT) activity as well as of dopamine content. These metabolic alterations were more pronounced in striatum than in cortex and were associated with diffuse morphological damage which began to be histologically evident already after 2 hrs. In addition, the exogenous administration of monosialoganglioside GM1 (30 mg/kg post-operatively) was capable of exerting a significant neuroprotective effect. These latter results strengthen the therapeutic potential of the ganglioside in clinical situations of cerebrovascular insufficiencies.

386.13

MEMBRANE FATTY ACID CHANGES IN PRIMARY & PERI-ISCHEMIC CORTICAL TISSUE FOLLOWING ACUTE GM1 GANGLIOSIDE TREATMENT. B. Hungund, V. Gokhale, A. Ortiz, S. E. Karpiak and S. P. Mahadik. Div. Neuroscience, NYSPI & Columbi U. (P&S), N.Y., N.Y.

We have reported that 24hrs after global cerebral ischemia (gerbil) individual membrane fatty acid (FA) losses are reduced with GM1 ganglioside treatment. Here we report this protective effect of GM1 in focal cerebral ischemia [permanent occlusion of the MCAo & ipsilateral common carotid artery (CCAo) & 1hr temporary contralateral CCAo]. Three groups of rats were used - ischemic /GM1 (10mg/kg, i.m.), ischemic/saline & sham/saline. Rats were sacrificed at 3&72hrs, 4&6wks. Ipsi- & contralateral brain areas (primary & peri-ischemic) were dissected out. Total membrane fractions were isolated & treated with ethanolic HCl to produce ethyl esters of individual FAs (palmitic; stearic; oleic; linoleic; arachidonic). FA ethyl esters were analyzed by GC; values were expressed as ugms/mg membrane protein. In the primary infarct, at 72hrs, slightly reduced levels of saturated FAs & significantly reduced levels of unsaturated FAs were found. These losses were slightly reduced in GM1 rats. *In the peri-ischemic area, large (25-35%) losses of unsaturated FAs were observed, but, a (100%) increase in all individual FAs in GM1 treated rats was found.* At 4&6wks, FA levels were similar in the peri-infarct area in all groups. At 3hrs, in all groups there were no changes in FA levels. In contralateral tissue in all groups, FA levels were unchanged. The data indicate that GM1 treatment restores membrane FA acid metabolism in ischemia. Supported in part by NINCDS (NS-2525856) & FIDIA Research Foundation.

386.14

EFFECTS OF GM1 ON SPREADING DEPRESSION. P. Miu, and K. Krnjević. Anaesthesia Research Dept., McGill University, Montreal, Canada. H3G 1Y6.

Spreading depression (SD) is a transient wave of depolarization resulting from disturbances in the composition of the intracellular and extracellular ions and water. Since exogenous monosialoganglioside (GM1) reduces Ca^{2+} current (Agopyan et al., Soc. Neurosci. Abstr., 1990) and enhances Na^+/K^+ pump activity (Vyskocil et al., Pflugers Arch., 403:1, 1985) we examined the possible protective role of GM1 in SD-like depolarization. In the present study, SD-like potential changes were triggered in hippocampal slices (34°-36°) by hypoxia and high $[K^+]_o$. A tungsten bipolar stimulating electrode was placed in the s. radiatum to elicit orthodromic responses in the CA1 region of s. pyramidale.

Extracellular recordings showed that prolonged application of GM1 (1 μM) may reduce the occurrence of SD (SD in 5 out of 10 slices) as compared with controls (SD in 6 out of 9 slices). In GM1 treated slices the latency and duration of SD were increased, and the post-hypoxic positive shift was augmented as compared to the control slices. Intracellular recordings showed that GM1 (1 μM) perfusion consistently induced cell depolarization associated with an increase in R_N. No significant changes were observed in hypoxia-induced hyperpolarization between the control and GM1 perfused slices. However, the ouabain-sensitive post-hypoxic hyperpolarization was greater during GM1 perfusion (-19.4 ± 1.9 mV, n=19) than control (-9.9 ± 1.0 mV, n=14).

The results suggest that exogenous GM1 may have a protective role in ischemia through its ability to reduce Ca^{2+} current and facilitate Na^+/K^+ pump.

Supported by Medical Research Council of Canada.

386.15

GM1 GANGLIOSIDE TREATMENT MAINTAINS CAPACITY OF ISCHEMIC TISSUE TO DEFEND AGAINST FREE RADICAL DAMAGE. S. P. Mahadik, J. Murthy*, A. Ortiz & S. E. Karpiak. Div. Neuroscience, NYSPI, and Columbia U., Physicians & Surgeons, New York, NY.

We have shown in rat, that cortical focal ischemia initiates a series of acute biochemical changes: cellular levels of Na+, K+, Ca2+; loss of plasma membrane Na+,K+- & Ca2+-ATPase; and increases in oxy-radicals, which reflect a general failure of plasma membrane function in primary & peri-infarct areas. Plasma membrane failure is thought to result from toxicity due to increased oxy-radical levels. This toxicity can be eliminated by the synergism of superoxide dismutase (SOD), catalase (CAT) & glutathione peroxidase (GSHPOD). We find these enzyme levels very low in normal tissue but increase (SOD=65%; CAT=250%; GSHPOD=120%) in the primary infarct, persisting up to 6 wks. Since irreversible membrane function loss occurs by 72 hrs, enzyme level increases are too slow to fully counteract oxy-radical toxicity. We have reported that acute GM1 ganglioside treatment of ischemia reduces membrane function losses. We have tested whether GM1 increases enzyme levels of oxy-radical metabolism *sooner.* Rats were treated with GM1 immediately after ischemia. Enzymes were assayed at 24,48&72hrs. No differences were found between GM1 treated & saline injected ischemic rats. But, *in GM1 treated rats, no loss of membrane fatty acids or* other membrane function parameters (supra) were seen. These data indicate that GM1 treatment protects membrane function and *preserves* normative physiological responses to defend against oxy-radical toxicity. Supported in part by NIH (NS-2525856) & FIDIA Research Foundation.

386.16

GM1 GANGLIOSIDE PREVENTS HYPOXIA-INDUCED NEUROTRANSMITTER CHANGES IN THE BRAIN OF THE DEVELOPING RAT. N.H. Neff, J. Stanisic, D. Krainc, and M. Hadjiconstantinou. Departments of Pharmacology and of Psychiatry, The Ohio State University College of Medicine Columbus, Ohio 43210 USA

Exogenously administered GM1 ganglioside (II^3NeuAc-GgOse$_4$Cer) promotes recovery of biochemistry, morphology and function following selective lesions of the nervous system. We have evaluated the consequence of administering GM1 on hypoxia-induced neurotransmitter changes in neonatal rats. Seven day old rats were exposed to hypoxic conditions (8% O_2, balance N_2) for 2.5 - 3 hr and the concentration of GLU, DA, DOPAC, 5-HT, 5-HIAA and ACh assayed in hippocampus, striatum and frontal cortex 15 min after the insult. Exposure for about 3 hr resulted in significant increases of GLU, DA, 5-HT and their metabolites in all three regions of brain. For ACh there was a decreased content in the three regions of brain. Treating rats daily with GM1, 50 mg/kg i.p., beginning on day 5 and 1 hr before and after exposure to hypoxia corrected these changes including the decrease of ACh. Furthermore, GM1 also decreased the mortality of the hypoxic rats. We conclude that GM1 may be of significant benefit for treating suspected neonatal hypoxia.

386.17

AUTORADIOGRAPHIC ANALYSIS OF REDUCED [45]Ca++ LOADING IN CORTICAL ISCHEMIA WITH GM1 GANGLIOSIDE TREATMENT. N. Hernandez, A. Ortiz, M. Durkin*, A.I. Barkai*, S.P. Mahadik & S.E. Karpiak. NY State Psych. Inst., & Columbia U. (Physicians & Surgeons), NY, NY.

GM1 ganglioside treatment reduces injury (edema, Na+, Ca++ increases) & behavioral deficits after CNS ischemia. We report here an autoradiographic study of [45]Ca++ uptake in ischemia, & the effects of GM1 treatment. Ischemia is induced by middle cerebral artery (MCAo) & ipsilat. carotid artery (CCAo) occlusion & 1hr temporary contralat. CCAo. Tissue is restricted to cortex; no tissue pathology occurs in subcortical areas. Our prior studies indicate that maximal Ca++ levels in primary & peri-infarct areas occur 72hrs after ischemia (basal Ca++ levels = 10umoles/gr d.w. tissue; Ca++ in primary infarct = 170umoles/gr d.w. tissue). To visualize the Ca++ increases, at 72hrs after ischemia, rats were injected (i.p.) with 100uCi [45]Ca++. After 5hrs rats were sacrificed & coronal brain slices were prepared for film autoradiography. Data indicate that GM1 reduces both [45]Ca++ loading and infarct size. *We also observed high levels of [45]Ca++ loading in subcortical structures (thalamic nuclei, tracts & the internal capsule).* These regions show no tissue pathology. High levels of Ca++ in tertiary brain areas may reflect injury processes heretofore not included in stroke pathology. These sucortical [45]Ca++ increases were markedly reduced using GM1. The ability of GM1 to reduce Ca++ increases in primary, peri-infarct and *tertiary injury areas* may be the basis for improved recovery seen on a spectrum of complex behavioral paradigms. Supported in part by grants from NINDCS (NS-2525856) and FIDIA Research Foundation.

386.18

NERVE GROWTH FACTOR TREATMENT OF CORTICAL FOCAL ISCHEMIA. A. Ortiz, J.S. MacDonall , S.P. Mahadik & S.E. Karpiak. Div. Neuroscience, NYSPI, & Columbia U., Coll. Physicians & Surgeons, N.Y., N.Y.

We are studying the role of NGF in recovery processes following cortical focal ischemia. We report here a study of intraventricularly (icv) injected NGF on neurological, sensory/motor, cognitive functions, and associated biochemical parameters (tissue ion concentrations, and changes in enzymes of oxy-radical metabolism) after cortical focal ischemia in rats. Previous reports indicate that NGF treatment after CNS lesion reduce behavioral dysfunction and protect selective neuronal damage. We have reported that rats with cortical focal ischemia [middle cerebral artery (MCAo) and common carotid artery occlusion (CCAo)], exhibit sensori-motor & cognitive dysfunctions, and, loss of plasma membrane function. Forty rats exposed to cortical focal ischemia and sham controls received 4 icv injections of either mNGF (supplied by Dr. E. Johnson: 2.5S: 10ug dissolved in 5ul saline) or saline, at 0,24,48&72hrs after surgery. After fourteen days, rats are exposed to a series of behavioral tests [days 14-40]. These include the tail-hanging, prehensile-traction, inclined plane and pole balancing tests. Spontaneous activity is also electronically monitored. In addition rats are trained and tested on a cognitive paradigm (spatial reversal learning). After all behavioral testing rats are sacrificed and tissue is analyzed for ion concentrations as well as levels of enzymes involved in oxy-radical toxicity (e.g. catalase). Initial data indicate that NGF treatment can ameliorate behavioral dysfunction associated with the ischemic injury. Supported in part by NINCDS (NS-2525856) & FIDIA Research Foundation.

386.19

PATTERN OF BEHAVIORAL DEFICITS FOLLOWING FOCAL CEREBRAL ISCHEMIA IN RATS: SENSORIMOTOR INTEGRATION AND COGNITIVE FUNCTION. C.G. Markgraf, B.E. Hurwitz, E. Morikawa*, W.D. Dietrich, M.D. Ginsberg, E. J. Green, P.M. McCabe and N. Schneiderman. Dep'ts. Psychology and Neurology , Univ. of Miami, Coral Gables, FL 33124.

Assessment of behavioral recovery of animals following focal cerebral ischemia is often difficult due to the subjective nature and limited scope of many behavioral tests. Using a number of behavioral tests, the present study quantified neurologic status in 6 Sprague-Dawley rats with proximal middle cerebral artery occlusion (MCAO) and 6 sham operated rats. MCAO leads to reproducible infarction of the striatum and overlying cortex. Five tests designed to measure forelimb reflexes and sensorimotor integration were given twice weekly for 1 week pre-operatively and 4 weeks post-operatively. ANOVA revealed no differences between the groups pre-operatively. Two days after MCAO, infarcted animals displayed significant neurological deficits compared to controls in abnormal posture when lifted by their tails; in foot faults on an elevated grid; on a bilateral assymmetry test of sensory neglect, and in contralateral limb placing to visual and tactile stimuli. Observed deficits were maximal on days 2-7 post-operatively and gradually recovered to control levels by day 28. Deficits were not seen in ipsilateral limb placing or on a test of strength on an inclined screen. Following the 30 days of neurologic testing, animals were given 10 days of testing in the Morris water maze, a test of spatial mapping ability. While ANOVA revealed that both MCAO and sham groups showed a decrease in latency to locate the hidden platform over days, the MCAO animals were consistently slower (latency=16.8 ± 1.5 sec. vs. 6.3 ± 1.5 sec. on day 10) and significantly less accurate in their initial heading than were controls. This battery of tests reveals a comprehensive picture of the deficits following MCAO and indicates that while sensorimotor integration recovers over 28 days, cognitive mapping deficits persist, paralleling results reported in human patients with striatal infarcts.

386.20

ARGININE VASOPRESSIN (AVP) AND EDEMA IN INTRACEREBRAL HEMORRHAGE. G.A. Rosenberg, O. Scremin, E. Estrada*, W.T. Kyner*. Neurology and Research Services, Veterans Medical Center, and Departments of Neurology, Physiology and Mathematics, University of New Mexico School of Medicine, Albuquerque, NM 87131

Vasopressin increases brain water and worsens brain edema. We used a model of edema after an intracerebral hemorrhage to determine the role of the AVP V_1-receptors. Adult rats had hemorrhages induced in the caudate-putamen by the infusion of 0.4 U (n=9) or 0.5 U (n=9) of bacterial collagenase. Two groups had 75 ng of a V_1- receptor antagonist, $d(CH_2)_5$,Tyr(Me),Arg, infused along with either 0.4 (n=9) or 0.5 U (n=9) of collagenase. Twenty-four hours after injection brain water and electrolyte contents were measured in 8 brain regions. Cerebral blood flow (CBF) was measured with the ^{14}C-iodoantipyrine autoradiographic method 24 hours after injection with either 0.5 U of collagenase (n=6) or saline (n=6). The V_1 antagonist significantly ($p<0.05$) reduced edema without altering CBF. AVP V_1-receptors are important in edema after an intracerebral hemorrhage.

ALZHEIMER'S DISEASE: CYTOSKELETON

387.1

TAU PHOSPHORYLATION IN HEAT-SHOCKED FEMALE AND MALE RATS AND CEREBRAL EXPLANTS. S. Ch. Papasozomenos and Y. Su . Dept. of Path., Univ. of Texas Med. Sch., Houston, TX. 77225.

We have previously shown that in the somatodendritic compartment of neurons tau becomes phosphorylated at or near the epitope of the antibody Tau-1 preventing its binding. To investigate whether the excessive tau immunoreactivity seen histologically in Alzheimer's disease might be stress-induced, we have studied control and heat-shocked (42°C for 15 min) 2-3-month-old female (5 pairs) and male (8 pairs) Sprague-Dawley rats and control and heat-shocked (42°C for 20 min) fetal rat cerebral explants. Six hr after heat shock, immunoblotts of SDS extracts from rat cerebrum and explants were analyzed by using Tau-1 (provided by Dr. L.I. Binder) and ^{125}I-protein A with and without pretreatment with alkaline phosphatase. In heat-shocked female rats there was: a) excessive phosphorylation of existing tau indicated by an increase in the ratio: phosphorylated + non-phosphorylated = total/non-phosphorylated Tau-1 epitope (4.04 vs 2.02, $p < 0.001$); b) a non-significant change of the total (cpm:5,051 vs 4,525, $p < 0.5$); and c) a marked reduction of non-phosphorylated tau (cpm:1,611 vs 2,627 $p < 0.01$). Similar but less pronounced changes were observed in age-matched male rats with the respective numbers being: a) 2.78 vs 2.42, $p < 0.05$; b) cpm:8,093 vs 10,376, $p < 0.5$; and c) cpm:2,960 vs 3,585, $p < 0.001$. On the contrary, in heat-shocked cerebral explants there was a marked increase in the amount of both total (cpm:8,032 vs 4,453, $p < 0.05$) and (cpm:5,455 vs 1,434, $p < 0.01$) tau and a pronounced decrease in the ratio: total/non-phosphorylated (1.50 vs 3.12, $p < 0.02$) due to excessive amounts of non-phosphorylated tau. This decrease in phosphorylation caused loss of the most slowly moving 60 Kd tau polypeptide. These findings suggest that tau plays a role(s) in the stressful response by altering its state of phosphorylation.

387.2

ANTIGENIC HETEROGENEITY OF NEUROFIBRILLARY TANGLES IN ALZHEIMER'S DISEASE W.Bondareff, C.M. Wischik,Dept. of Psychiatry, University of So. California, Los Angeles, and M.R.C. Laboratory of Molecular Biology, Cambridge, England.

Antibodies directed against three regions of tau have been used to distinguish two populations of neurofibrillary tangles. Intracellular tangles, immunolabelled by polyclonal antibodies directed against N- and C-termini of tau were shown to be antigenically distinct from extracellular tangles, immunolabelled by monoclonal antibodies against antigens in the triple repeat region of the molecule. Exposure of the latter appeared to be associated with the proteolysis of paired helical filaments occurring in the extracellular compartment. In the CA1 region of hippocampus, a subset of extracellular tangles were immunolabelled also by monoclonal antibodies against B-amyloid in some patients. These patients were characterized by a severe degree of pyramidal cell loss and a high number of extracellular in CA1.

387.3

TRANSFECTION OF cDNAs ENCODING DIFFERENT ISOFORMS OF HUMAN TAU. M.M.S. Lo[1], A.W. Fieles*[1], C.B. Caputo[1], L.G. Sygowski[1], C.W. Scott[1], and M. Goedert*[2]. [1]ICI Americas, Wilmington, DE 19897, and [2]MRC Laboratory of Molecular Biology, Cambridge CB2 2HQ, U.K.

Tau proteins are low molecular weight microtubule-associated proteins normally expressed throughout the mammalian nervous system. Tau is also found in the protease-resistant core of the paired helical filament, which forms the bulk of the neurofibillary tangles found in neuropathologic brain samples of Alzheimer's disease. The genes encoding for six different tau isoforms, cloned from human cDNA libraries (Goedert et al., Neuron 3:519, 1989), were subcloned into expression plasmids and transfected into 3T3 fibroblasts and PC12 cells. High levels of transient type I tau (3 repeat) expression were detected by immunofluorescence assays with anti-tau antibodies in 3T3 cells. In most cases tau protein was in either small intense patches within cell bodies or in processes. In one case intense nuclear staining was detected. Stable PC12 transformants, cotransfected with the *neo* gene and selected with G418, showed vey low levels of tau expression In contrast, moderate levels of tau expression was detected in stably transformed 3T3 cells. Tau staining was found in the cytoplasm and in very long, thin, and branched "neurite-like" extensions. The stability of microtubules formed within these cell lines (and others transfected with plasmids encoding for the different human tau isoforms) will be tested by treatment with microtubule-depolymerization drugs such as colchicine and nocodazole.

387.4

ALZHEIMER BRAIN FRACTIONS BEARING PHF CONTAIN WHEAT GERM AGGLUTININ BINDING ACTIVITY. L. McLaughlin, F. P. Zemlan, and G. E. Dean. Department of Mol. Genetics, Biochemistry, and Microbiology, Univ. of Cincinnati College of Medicine, Cincinnati, OH 45267-0524.

Alzheimer brain neurofibrillary tangles, composed primarily of paired helical filaments (PHF), have been previously reported (Szumanska et al, 1987. Acta Neuropath., Berl. 73:1-11) to bind wheat germ agglutinin (WGA). Fractions of Alzheimer brain extracts highly enriched in PHF have been separated by SDS PAGE, electroblotted, and the blots probed with WGA coupled to horseradish peroxidase (HRP). Alzheimer-specific reactivity was observed at positions corresponding to M_R's of 85, 74, 39, and 37 kD, lacking in similarly obtained fractions from normal brain. When probed with antibodies reactive with purified PHF proteins, immuno-reactivity was observed at M_R positions corresponding to 85 and 37 kD in both Alzheimer and normal samples, suggesting that certain normal proteins are inappropriately glycosylated in Alzheimer brain tissue.

387.5

PHOSPHORYLATED TAU IN ALZHEIMER'S DISEASE PAIRED HELICAL FILAMENTS. L. Baum, E. Masliah, K. Ueda*, D. Iimoto, T. Saitoh, UCSD, School of Medicine, Dept. of Neurosciences, M-024, La Jolla, CA 92093.

Paired helical filaments (PHF) are found in the degenerating neurites surrounding amyloid plaques, and in the neurofibrillary tangles (NFT) of Alzheimer's disease (AD). A major PHF constituent is abnormally phosphorylated tau, a microtubule-associated protein. Phosphorylation of microtubule-associated proteins is known to reduce their ability to promote microtubule polymerization. One could speculate that abnormal phosphorylation of tau may disrupt the neuronal cytoskeleton, contributing to cell injury and death.

Either acid or alkaline phosphatase treatment of AD brain sections enhances NFT staining with anti-tau monoclonal antibody (mAb) Tau-1, suggesting that the Tau-1 epitope is masked by phosphorylation in AD NFT. Phosphatase treatment of immunoblots of AD brain pellet fractions and of purified PHF also enhanced Tau-1 binding to a doublet of ~60 kD mol. wt.

NFT is also stained by Alz-50, a mAb that preferentially stains AD brain and is not sensitive to alkaline phosphatase treatment. However, we found that acid phosphatase reduces Alz-50 staining of NFT. Furthermore, phosphoserine, but not serine or phosphothreonine, diminishes Alz-50 staining of NFT, pointing to phosphorylated serine(s) on tau as the epitope for Alz-50.

The tau sequence contains at least four theoretical serine phosphorylation sites for casein kinase II (CK-II), and tau is phosphorylated by CK-II. We previously found that anti-CK-II immunoreactivity is localized to NFT, and that purified CK-II binds NFT on AD brain sections. We are now investigating the possibility that CK-II may contribute to the phosphorylation of tau at Tau-1 or Alz-50 epitopes.

387.6

A NOVEL NEUROFILAMENT KINASE. H.M.Roder* and V.M.Ingram. Dept. of Biology, Mass. Inst. of Tech, Cambridge, MA 02139

The two heavier subunits of mammalian Neurofilaments (NF) (Mw 68, 160, 200kD) contain repeats of a Lys-Ser-Pro-sequence, which are extensively phosphorylated for as yet unknown reasons. We are purifying and characterizing a corresponding kinase not associated with the cytoskeleton by conventional 32-P-assays and a novel immunoassay. This kinase might act also on an identical site in tau protein, which seems to be phosphorylated and occurs in the carboxy-terminal portion of the molecule incorporated into the hard core of Paired Helical Filaments in Alzheimer's Disease.

A novel immunoassay based on completely dephosphorylated bovine NF-triplet and monoclonal antibodies specific for the phosphorylated Lys-Ser-Pro-sequence paved the way to partially characterize and purify a kinase activity from an ammonium-sulfate fractionated bovine brain supernatant. The activity survives chromatography only on a few media in the presence of Mg ions and ATP. Gel filtration suggests a Mw around 60kD with a probable proteolytic fragment at 40kD. All NF-subunits, native or dephosphorylated, are substrates to a varying extent. Significant levels of PO_4 are incorporated into dephosphorylated NF 160 and 200 with shifts to higher apparent Mw (SDS-PAGE) after 18 hrs of incubation. The properties of the activity, such as lack of association with cytoskeleton proteins, stability, inhibition by ionic strength and kinetic parameters, identify it as a new kinase.

387.7

EXPRESSION OF PAIRED HELICAL FILAMENT ANTIGEN IN ALZHEIMER'S DISEASE AND ISCHEMIA. M.M. Robertson, N.P. Galletly, S. Greenberg, P. Davies and C.B. Saper. Depts. of Pharm. & Physiol. Sci. and Neurology, Univ. of Chicago, Chicago, IL 60637 and Dept. of Pathology, Albert Einstein Medical College, Bronx, NY 10461.

PHF-1 is a monoclonal antibody raised against a denatured preparation of paired helical filaments from brains of patients with Alzheimer's disease (AD) (Greenberg and Schein, Soc. Neurosci. Abstr. 15:1083). We mapped PHF-1 immunoreactivity in the brains of six patients with AD, four controls without neurological disease and one 22 y.o. patient with a transient global ischemic event. In the normal brains, only occasional neurons stained, mainly in a fibrillar pattern. In the AD brains, there was fibrillar staining of cell bodies and associated dendrites, dystrophic axons in fiber pathways and dystrophic neurites in neuritic plaques. The areal and laminar staining pattern was similar to the AD pattern seen with another monoclonal antibody, ALZ-50, as well as the AD neurofibrillary degeneration visualized using thioflavin-S staining, in adjacent sections from the same brains. In the ischemic brain, PHF-1 stained neurons in a granular, cytoplasmic pattern in the medial temporal lobe only. The densest staining was in the CA1 region of the hippocampal formation, and occasional neurons in the entorhinal cortex appeared to contain immunoreactive tangles. Our observations suggest that PHF-1 stains an epitope present on paired helical filaments in AD that is also present on a protein expressed during cellular ischemic stress.

387.8

***IN SITU* HYBRIDIZATION OF CALCIUM/CALMODULIN DEPENDENT PROTEIN KINASE II AND TAU mRNAs IN ALZHEIMER'S DISEASE.** V.H. Mah, T.A. Eskin and G.A. Higgins. Univ. Rochester., Rochester NY 14642 and Gerontology Res. Ctr., NIA/NIH, Baltimore, MD 21224.

Abnormal phosphorylation of the microtubule-associated protein tau in Alzheimer's Disease (AD) may be caused by altered expression of calcium/calmodulin dependent protein kinase II (cam kinase II). We performed *in situ* hybridization studies in the human CNS to examine the expression of cam kinase II and tau mRNAs, and correlate their distribution with the presence of neurofibrillary tangles and neuritic plaques in AD. In isocortex, the laminar-specific pattern of neuronal hybridization (layers III, V-VI) was similar for both mRNAs. In some regions of association cortex, cam kinase II mRNA levels were higher than tau in layer II. In allocortex, layer II contained the highest abundance of both mRNAs. The hippocampal formation, amygdala, and nucleus basalis contained high levels of both mRNAs. In AD, areas which appeared to contain viable neurons but had large numbers of plaques and tangles showed similar levels of both tau and cam kinase II mRNAs as in control cases. Future studies will examine the expression of different subtypes of these mRNAs.

387.9

A PROGRESSIVE APPEARANCE OF A TAU-RELATED EPITOPE IN NEURITES CHARACTERIZES THE EVOLUTION OF ALZHEIMER'S DISEASE PATHOLOGY. R. Mena*[1], C.M. Wischik*[2], M. Novak*[3], C. Milstein*[4], A.C. Cuello[1]: [1], Department of Pharmacology and Therapeutics, McGill University, Montreal, Quebec, H3G 1Y6, Canada; [2], Department of Psychiatry, Cambridge University, Hills Road, Cambridge, CB2 2QQ, England; [3], Slovak Academy of Sciences, Bratislava, Czechoslovakia; [4], Laboratory of Molecular Biology, Medical Research Council, Cambridge, CB2 2QH, England.

The paired helical filament core (PHFc) present in Alzheimer's disease (AD) brains, contains the microtubule binding domain of tau (Wischik et al., PNAS (USA) 85:4884, 1988). Using the monoclonal antibody 6.423, which specifically recognizes this tau-related epitope of the PHFc, we studied frontal cortex of AD cases with short and long histories of dementia. In all cases, 6.423 mAb recognized both intracellular and extracellular neurofibrillary tangles (NFTs). However, in cases with longstanding dementia, a dense network of immunoreactive (IR) thread-like structures was observed in addition to the NFTs. The appearance of such structures seemed to lag behind that of IR NFTs. The IR thread-like material may represent fragmentation of the tangle and/or aberrant sprouting of affected neurons and, therefore, reflect the progression of AD pathology.

(Supported by MRC Canada and Medicorp, Canada.)

387.10

ALZHEIMER NEUROFIBRILLARY TANGLES CONTAIN 2.1±0.2 nm FILAMENTS THAT RESEMBLE ISOLATED BOVINE TAU PROTEIN G.C. Ruben*, °K. Iqbal, °I. Grundke-Iqbal*, °H.M. Wisniewski*, @T.L. Ciardelli* and +J.E. Johnson, Jr. Dept of Biol. Sci., Dartmouth College, Hanover, NH 03755; °Inst. for Basic Res. in Develop. Disabil., Staten Island, NY 10314; Dept of Pharm., Dartmouth Med. Sch., Hanover, NH 03756; Dept of Integ. Biol., UC, Berk., CA and Dept of Neurosci., Stanford Res. Inst., Menlo Park, CA

High resolution transmission electron microscopy (TEM) has shown that neurofibrillary tangles (NFT) contain in addition to paired helical filaments (PHF) numerous 2.1±0.2 nm filaments. These 2.1±0.2 nm filaments are triple-stranded left-handed helical structures composed of three 1.0±0.2 nm strands. These tangle filaments have been compared to purified bovine tau and found to have the same structure as judged by high resolution TEM. The reported amino acid sequence of human and bovine tau have been computer processed to predict secondary structure. Within the constraints imposed by the images, the secondary structure models and other structural information have been used to calculate tau's maximum and minimum length. This work suggests that each ~1.0 nm strand is a tau sequence and that the ~2.1 nm filament is composed of three tau sequences (tau_3). Bovine tau length measurements point out that each tau_3 filament is many times longer than a fully stretched tau monomer. These measurements indicate that tau_3 forms long polymers, $(tau_3)_n$. A inverse temperature transition has been found in the circular dichroism spectrum of tau indicating that its structure is less ordered below 20°C and more ordered at 37°C. This phenomena helps explain how tau can reconstitute microtubules above 30°C and why tau is unable to reconstitute microtubules below 20°C. The inverse temperature transition is also found in the protein elasin which aggregates at body temperature. If tau produced in Alzheimer victims was more hydrophobic than normal tau it could aggregate into tangles similar to the mechanism of elastin aggregation.

387.11

CHARACTERISTICS OF THE TAU THAT IS BOUND TO ALZHEIMER PAIRED HELICAL FILAMENTS. C.B.Caputo, C. Wischik*, W.F. Brunner*, C.W Scott* and A.I. Salama. ICI Pharmaceuticals Group, ICI Americas Inc., Wilmington, DE 19897.

Tau protein is known to be a component of Alzheimer paired helical filaments. The present study investigated the structural characteristics of the tau isolated from pronase treated PHFs (Wischik et al., PNAS,85:4506,1988). Antibodies with tau epitopes N terminal to the tubulin binding region of tau failed to react to pronase treated PHFs. Three of 4 antibodies to pronase treated PHFs reacted only after formic acid or guanidine treatment of PHFs. Two of these antibodies (751 & 795) reacted to tau isolated from normal brain whereas the other 2 (423 & 728) only recognized PHF-derived tau. The phosphate dependent antibody SMI34 also recognized tau from PHFs. The C-terminus of tau was present after pronase treatment based upon reactivity of an antibody raised to a peptide from the C terminus of tau. In conclusion, the C terminus including the whole tubulin binding region appears to be protected from proteolysis by an interaction with other PHF components & may contain phosphorylated sites & other modifications or conformations that are not always present in tau from normal brains.

387.12

MICROTUBULE REASSEMBLY IN ALZHEIMER BRAIN: AN ELECTRON MICROSCOPIC STUDY. E.M. Grollman, D.L. Rapoport* and J.A. Olschowka. Department of Neurobiology & Anatomy and Department of Neurology, University of Rochester Medical Center, Rochester,NY 14642.

Microtubules, vital cytoskeletal structures common to all eucaryotic cells, participate in many important neuronal functions including intracellular compartmentalization, neurite outgrowth and axonal transport. The microtubule-associated protein (MAP), tau, a normally occuring component of axonal microtubules has been shown to have unusual features in Alzheimer's Disease (AD) brain and may be implicated in the pathophysiology of AD. For example, Flament and Delacourte (FEBS. 247: 213, 1989) have reported AD specific isoforms of tau and markedly abnormal patterns of tau immunostaining have been demonstrated in AD brain (Kowall & Kosik, Ann. Neurol. ,22:639, 1987). Of greatest interest is the substantial amount of evidence pointing to tau as a component of the neurofibrillary tangle (NFT) (eg Woods et.al., PNAS. 83:4040,1986; Kosik et.al.,PNAS. 83:4044, 1986; Goedert et.al., PNAS. 85:4051,1988).

Though it has been reported that reassembly of microtubules from AD brain is impaired, possibly because of the presence of a dysfunctional form of tau (Iqbal et.al., Lancet. 2:421, 1986), we were able to reassemble microtubules *in vitro* from homogenates of superior frontal gyrus obtained from AD and control brains (range of postmortem delays: 4 to 14 hours). These preparations were examined electron microscopically after negative staining with 1% uranyl acetate. No intrinsic differences between AD and control were observable by this method. Ongoing research is focused on the antigenic characteristics of the reassembled microtubule in AD.

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387.13

ACTIONS OF CALCIUM ON THE CYTOSKELETON AND TAU IN CULTURED HUMAN CEREBRAL CORTICAL NEURONS. M. P. Mattson, G. Perry and M. G. Engle*. Center on Aging and Dept. of Anatomy & Neurobiol., Univ. of Kentucky, Lexington, KY 40536; Inst. of Pathology, Case Western Reserve Univ., Cleveland, OH 44106.

Abnormalities in the neuronal cytoskeleton are hallmarks of an array of neurodegenerative disorders (ND). Some of the changes observed in the human brain (e.g., paired helical filaments [PHF] and neuritic plaques) have not been observed in common laboratory species. We have therefore begun to directly examine cell cultured fetal human cerebral cortical neurons (see Rychlik et al., this meeting). Since considerable circumstantial evidence supports the involvement of altered calcium homeostasis in NDs (*Mech. Aging Dev.* 50:103), we studied cytoskeletal changes associated with calcium influx using immunocytochemistry and electron microscopy. Axons in untreated neurons were dominated by parallel arrays of microtubules. Exposure to calcium ionophore A23187 resulted in a dramatic loss of microtubules, accumulations of microfilaments, and the appearance of 12-15 nm straight filaments. PHF-like structures were occassionally observed. Tau immunoreactivity increased in neurons exposed to A23187, but Western blots indicated that tau levels (as well as actin and tubulin) actually decreased as a result of calcium influx. Colchicine (but not cytochalasin D) also increased tau immunoreactivity, by a calcium-independent mechanism, suggesting that depolymerization of microtubules may be the trigger for the altered disposition of tau, and its accumulation in the straight filaments and PHF that characterize neurofibrillary tangles. Immunoelectron microscopy is now being used to more directly determine the fate of tau consequent to calcium influx. (supported by a PSP grant and an ADRDA Faculty Scholar Award to MM).

EPILEPSY: BASIC MECHANISMS II

388.1

FADING OF RECURRENT INHIBITION IN THE RAT DENTATE GYRUS IS MEDIATED BY $GABA_B$ RECEPTORS. David D. Mott and Darrell V. Lewis. Depts. of Pharmacology, Pediatrics (Neurology) and Neurobiology, Duke University Medical Center, Durham, N.C. 27710.

Recent studies have shown that the $GABA_B$ receptor agonist, baclofen, suppresses recurrent inhibition in the dentate gyrus and facilitates the development of long term potentiation. Here, we present evidence showing that released GABA, acting on $GABA_B$ receptors, causes fading of recurrent inhibition.

Fading of inhibition was studied in the dentate gyrus of the rat hippocampal slice. Stimulation was delivered to the mossy fibers (MF) in stratum lucidum of CA3b and to the perforant path (PP) in the white spot on the distal side of the hippocampal fissure. PP stimulation evoked a population spike (PS) superimposed on a population epsp recorded extracellularly in the granule cell layer. MF stimulation inhibited a subsequent PP-evoked PS through $GABA_A$ receptor-mediated recurrent inhibition. A single conditioning MF stimulus depressed subsequent recurrent inhibition. This depression of inhibition was maximal 200 msec after the MF conditioning stimulus and lasted for up to 2 sec. The $GABA_B$ receptor antagonists, phaclofen (1 mM) or 2-OH saclofen (400 uM), reduced this depression of inhibition.

Intracellular recordings from granule cells demonstrated that MF stimulation evoked a biphasic ipsp with an early component sensitive to picrotoxin (50 uM) and a late component sensitive to 2-OH saclofen (400 uM). Paired MF stimulation caused a depression of the ipsp evoked by the second stimulus. This depression was strongest when the interstimulus interval was 200 msec and was antagonized by 2-OH saclofen (400 uM).

We suggest that GABA, released by the conditioning stimulus, acts on $GABA_B$ receptors causing a depression of recurrent inhibition.

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388.2

AN AMPA/KAINATE RECEPTOR-MEDIATED COMPONENT TO EPILEPTIFORM ACTIVITY IN THE KAINIC ACID LESIONED HIPPOCAMPUS. L.H.Simpson* and H.V. Wheal, Department of Neurophysiology, University of Southampton, Bassett Crescent East, Southampton, SO9 3TU. UK.
(Sponsored by the Brain Research Association, UK).

In previous electrophysiological studies of synaptic activity in the kainic acid-lesioned rat hippocampus, we have demonstrated graded epileptiform bursting activity that is mediated, at least in part, by NMDA-receptors (Ashwood, T.J. & Wheal, H.V. (1986) Neurosci. Letts. 67, 147-152; Wheal & Turner, (1988) Soc. Neurosci. 14, 709). The purpose of the present study was to pharmacologically determine the contributions of AMPA/kainate-receptors to suprathreshold bursting activity in this model. Both intra- and extracellular studies were performed in the CA1 cells of lesioned hippocampal slices.

The AMPA/kainate-receptor antagonist, CNQX (2uM), completely blocked the evoked action potential in CA1 pyramidal cells in control slices. In contrast, in experiments on lesioned slices, CNQX failed to completely block the evoked burst. The response was reduced to one or two spikes with an increase in first spike latency from 2.8 ms (±0.2, SEM, n=10) to 4.8 ms (±0.9, SEM, n=8). The CNQX insensitive component of the burst was blocked by subsequent perfusion with a selective NMDA-receptor antagonist (D-AP5; 10uM). These data suggest that both NMDA- and AMPA/kainate-receptors contribute to the suprathreshold epileptiform burst in the kainic acid lesioned hippocampus.

Supported by the Wellcome Trust.

388.3

NMDA-RECEPTOR MEDIATED EPILEPTIFORM ACTIVITY IN ORGANOTYPIC CULTURES OF RAT HIPPOCAMPUS. T. Sakaguchi, C. Shin, and J. O. McNamara. Duke and VA Med. Ctr., Durham, N.C. 27705.

Organotypic cultures of hippocampus (HPC) provide an excellent longlasting *in vitro* system in which structure and intrinsic connectivity are relatively preserved. Many reports of neuronal activity in these cultures document more epileptiform activity than acute adult HPC slices. To study the excitability of populations of HPC neurons, we used extracellular field recording technique to characterize the organotypic HPC culture electrophysiologically.

Organotypic cultures were prepared from 6-day-old rat HPC according to the methods of Gähwiler. Recordings were made in cultures of 5-35 DIV in a submersion chamber at 36°C with ACSF (95% O_2, 5% CO_2) perfused at 2 ml/min. Extracellular field recordings were made from the pyramidal layer in CA1, with stimulation of Schaffer collaterals in CA3.

Spontaneous interictal activity was uncommonly observed (< 15% of cultures) and spontaneous ictal events were never observed. Evoked CA1 field response showed EPSP with orthodromic population spike (PS) which was often followed by a burst of low amplitude PSs. Bath-applied 50 μM D-APV suppressed the late component of the EPSP and the superimposed bursts. Addition of up to 20 μM CNQX suppressed the remaining field EPSP and PS. Removal of Mg^{++} from the bath induced evoked and/or spontaneous epileptiform activity (interictal and/or ictal) in CA1 which was also suppressed by 50 μM D-APV.

This method of slice culture provides a network virtually devoid of spontaneous epileptiform activity, yet this activity is readily inducible by removal of Mg^{++}. These electrophysiologic features, together with the advantage of long term survival, make the organotypic HPC culture a useful tool for studying the **development** of epileptiform activity in a simplified neural system.

388.4

STIMULUS TRAIN INDUCED SEIZURES IN ORGANOTYPIC CULTURES OF RAT HIPPOCAMPUS. C. Shin, T. Sakaguchi, and J. O. McNamara. Epilepsy Research Laboratory, Duke and VA Medical Centers, Durham, NC 27705.

Electrographic seizures (EGS) induced by stimulus trains in slices of adult rat hippocampus (HPC) have provided an **acute** *in vitro* model of epileptogenesis (Stasheff et al., Science, 1989). Development of a **chronic** *in vitro* model would permit study of longterm plasticity in epileptogenesis. Organotypic cultures of HPC, with long survival time, may serve the purpose. We therefore sought to determine whether stimulus train could evoke EGS in this preparation.

Organotypic HPC cultures (12-36 DIV) were studied in a submersion chamber at 36 °C with standard ACSF (95% O_2, 5% CO_2) bath. Extracellular field recordings were made in CA1 pyramidal layer, with stimulation of Schaffer collaterals in CA3. Once orthodromic responses to single shocks were stable, 2 sec trains of 60 Hz rectangular pulses (150-1400 μA) were delivered every 10 min.

Stimulus trains reliably induced EGS with the typical pattern of initial high frequency ("tonic") discharge, followed by slower frequency bursts ("clonic"). Induced EGS were of relatively long duration (5-19 sec), even on the first stimulation. With repeated stimulation, however, the duration of EGS did not reliably increase. Application of the NMDA receptor antagonist, D-APV (50 μM), modified the pattern of the EGS without consistently altering its duration. Even when D-APV was applied prior to the first stimulus, fully developed EGS appeared.

The reliable induction of EGS by stimulus trains offers considerable promise for development of a chronic *in vitro* model of epileptogenesis. The occurrence of a fully developed EGS on the first stimulus and the relative insensitivity to D-APV suggest that this preparation is hyperexcitable prior to stimulation. Study of various factors regulating this hyperexcitability during culturing process may shed light on mechanisms of epileptogenesis and on the ongoing plasticity in culture.

388.5

THE ROLES OF EXCITATORY AMINO ACID RECEPTORS IN EPILEPTIFORM ACTIVITY INDUCED BY 4-AMINOPYRIDINE IN RAT AMYGDALA SLICES. P.W. Gean. Dept. of Pharmacology, College of Med. National Cheng Kung Univ. Tainan City, Taiwan, R.O.C.

The effects of excitatory amino acid receptor antagonists on epileptiform activity induced by 4-aminopyridine (4-AP) were studied in rat amygdala slices using intracellular recording techniques. Five to ten minutes after switching to 4-AP containing solution, spontaneous epileptiform bursts were observed in 35 out of 45 slices studied. Superfusion with kynuretic acid reversibly reduced the duration of the spontaneous bursting discharges in a dose-dependent manner. The IC_{50}, estimated from the graph of the concentration-response relationship, was approximately 130 μM. In addition, CNQX which is a specific non-N-methyl-D-aspartate (NMDA) receptor antagonist blocked the spontaneous and evoked epileptiform bursting. In 11 out of 15 neurons tested, there was a residual depolarizing component remained. This depolarizing component was reversibly blocked by specific NMDA receptor antagonist, DL-2-amino-5-phosphonovaleate (DL-APV). Relative to CNQX-sensitive component, the DL-APV-sensitive component is much smaller in amplitude and shorter in duration indicating that NMDA receptor plays only a minor role in this process. These data suggest that the generation or propagation of spontaneous epileptiform events induced by 4-AP in the neurons of basolateral amygdala is mediated by excitatory amino acids and that activation of non-NMDA receptors is of primary importance.

388.6

DENTATE MOSSY FIBER SPROUTING IN KAINATE-TREATED RATS LEADS TO EPILEPTIFORM ACTIVITY WHEN SYNAPTIC INHIBITION IS DEPRESSED J. Cronin, A. Obenaus, C.R. Houser and F.E. Dudek Mental Retardation Res. Ctr. and Dept. of Anatomy and Cell Biology, UCLA Sch. of Med., Los Angeles, CA 90024.

Sprouting of the mossy fibers (MFs) into the inner molecular layer of the dentate gyrus of kainate-treated rats may cause formation of a recurrent excitatory circuit, which could hypothetically cause epileptogenesis. Extracellular recordings from slices of kainate-treated rats suggested hyperexcitability to hilar stimulation in normal solution (Tauck, D.L. and Nadler, J.V., J. Neurosci. 5:1016, 1985). We have used simultaneous intracellular and extracellular recording in hippocampal slices to examine the electrophysiology of dentate granule cells that have undergone MF sprouting. In normal solution, slices with MF sprouting usually had antidromic and synaptic responses to electrical stimulation of the hilus and perforant path that were similar to those from control animals. However, when inhibition was reduced with the $GABA_A$ receptor antagonist, bicuculline, low-intensity hilar stimulation evoked delayed bursts of action potentials. Spontaneous bursts of population spikes, which were synchronous with intracellular action potentials superimposed on a depolarization, also occurred in bicuculline-treated slices. The delayed bursts to weak hilar stimulation and the spontaneous bursts in bicuculline were not observed in slices from kainate-treated rats without MF sprouting or in control animals. Thus, MF sprouting into the inner molecular layer of dentate granule cells can contribute to epileptiform activity when synaptic inhibition is reduced.

388.7

SYNCHRONOUS ACTIVITY INDUCED BY 4-AMINOPYRIDINE (4AP) IN THE CA3 SUBFIELD OF THE RAT IMMATURE HIPPOCAMPUS. **M. Avoli, V. Tancredi, C. Zona and Y. Fueta*. MNI and McGill Univ, Montreal, QC, Canada.**

Field potential recordings were made in the CA3 area of hippocampal slices obtained from 10 to 30-day-old rats during perfusion with medium containing the convulsant 4AP (50 μM). Three types of spontaneous potentials were observed. The first one was a 0.4-1.1 s long potential (frequency of occurrence: 0.6-1.3 Hz) that resembled an epileptiform interictal event. The second type was reminiscent of an ictal epileptiform discharge, lasted 10-35 s and recurred every 40-100 s. The third potential was of opposite polarity to the other two types, occurred every 10-100 s and was usually followed by the ictal discharge. Application of NMDA receptor antagonists did not modify any of the three events while the non-NMDA receptors antagonist CNQX blocked both interictal and ictal discharges but not the potential of opposite polarity. The latter was however blocked reversibly by the $GABA_A$ receptor antagonist bicuculline methiodide. Thus in addition to epileptiform activity of interictal and ictal type that is dependent on non-NMDA conductances, 4AP also induces in the immature rat hippocampus a synchronous event that is due to the activation of the $GABA_A$ receptor.

Supported by The Hospital for Sick Children Foundation, the Savoy Foundation and the MRC of Canada (MA-8109).

388.8

NMDA-ACTIVATED CONDUCTANCES AND EPILEPTIFORM ACTIVITY INDUCED BY REPETITIVE STIMULI IN THE CA3 SUBFIELD OF RAT IMMATURE HIPPOCAMPUS PERFUSED WITH 4-AMINOPYRIDINE (4AP). **V. Tancredi, C. Zona, and M. Avoli. Univ "Tor Vergata", Rome, Italy; MNI and McGill Univ, Montreal, QC, Canada.**

When 4AP (50 μM) is applied to hippocampal slices obtained from 2 to 10-day-old rats, spontaneously occurring epileptiform activity is rarely seen in the CA3 subfield. In this experimental condition however, a short train of orthodromic stimuli elicits a prolonged electrographic seizure (20-40 s) that is then followed by spontaneous epileptiform events that recur regularly for several tens of min (up to 2 hours) in the absence of any further electrical stimulus. This type of epileptiform activity is not influenced by the subsequent application of the NMDA receptor antagonists CPP or MK-801 while being blocked by CNQX. Perfusion of non-stimulated hippocampal slices with medium containing CPP and 4AP does not affect the train-induced electrographic seizure response, but prevents the appearance of the spontaneous epileptiform discharges. Thus at this stage of brain maturation, NMDA receptors do not appear to play a role in the generation of 4AP-induced epileptiform activity while being important for establishing the long-lasting state of hyperexcitability that is associated with the occurrence of spontaneous epileptiform discharges.

Supported by The Hospital for Sick Children Foundation, the Savoy Foundation and the MRC of Canada (MA-8109).

388.9

AXONAL REORGANIZATION IN THE MOUSE HIPPOCAMPUS AFTER INTRAVENTRICULAR KAINIC ACID RC Green, SM Hersch, HD Rees, RAE Bakay Departments of Neurology and Neurosurgery, Emory University School of Medicine, Atlanta, GA 30322

Hippocampal sclerosis is the most common pathological finding in patients who undergo temporal lobe resection for medically intractable temporal lobe epilepsy. This characteristic pattern of pyramidal cell loss has been associated with reorganization of both Timms-positive fibers (Sutula et al, 1989) and acetylcholinesterase (AChE)-positive fibers (Green et al, 1989) in the human. In animals, intraventricular kainic acid (KA) causes seizures and excitotoxic damage to the hippocampus producing a pattern of cell loss similar to that seen with human hippocampal sclerosis. KA-induced cell loss is associated with aberrant AChE and Timms staining in the molecular layer of the rat dentate gyrus, suggesting axonal sprouting and reinnervation of deafferented neurons (Nadler et al, 1980).

In this study, male C57BL/6 mice, aged 2-4 months, were treated with bilateral intraventricular injections of KA (either 0.1 or 0.3 µg/side). After two weeks, mice were killed and their brains were processed for Nissl staining along with either AChE or Timms staining. Mice treated with KA revealed discrete areas of pyramidal cell loss with relative sparing of dentate granule cells and extra-hippocampal areas, including the septum and entorhinal cortices. The distribution of Timms staining did not differ from that found in controls, but AChE histochemistry revealed increased staining of the stratum oriens and stratum pyramidale in the areas of cell loss only. This is an unusual pattern of reinnervation since AChE staining was enhanced in a region where target neurons were lost without obvious deafferentation. One explanation is that surviving pyramidal neurons may be reinnervated by AChE-positive afferents in response to the loss of connections from adjacent neurons that are destroyed within the same sector.

388.10

INCREASED SPONTANEOUS EXCITATORY INPUT TO CA3 NEURONS DURING OVERHYDRATION OF HIPPOCAMPAL SLICES. V. Saly and R.D. Andrew, Anatomy Department, Queen's University, Kingston, Ontario K7L 3N6.

The clinical signs of rapidly developing overhydration commonly include generalized tonic-clonic seizure. Recent studies in hippocampal slices have indicated that field potentials (and therefore their synchronizing field effects) increase in hyposmotic conditions. As in CA1, we found the evoked anti- and orthodromic CA3 population spike was inversely proportional to the osmolality. The change occurred within 2 or 3 min, presumably coinciding with cellular swelling. However longer term effects were also observed in 9 CA3 cells recorded intracellularly for 1-3 hr. While resting potential, input resistance and spike threshold were unchanged by -40 mOsm saline over 10-40 min, spontaneous EPSPs increased in amplitude (7 cells). Sychronized input (represented by compound EPSP/IPSPs) was also induced (4 cells). These effects were reversed over 15-30 min in normosmotic saline. Hyperosmotic saline (+40 mOsm mannitol) had the opposite effect, reducing spontaneous EPSPs and any remaining EPSP/IPSPs (6 of 6 cells). In 2 of 2 cells tested, spontaneous interictal bursts were blocked by mannitol.

Thus both random and synchronized excitatory synaptic input to CA3 cells can evolve over tens of minutes during overhydration, suggesting a synaptic mechanism (in addition to field effects) whereby hyposmolality promotes excitation.

388.11

POSTNATAL DEVELOPMENT OF Na,K-ATPASE-MEDIATED HYPERPOLARIZATIONS IN RAT HIPPOCAMPAL CA1 NEURONS. A. Fukuda and D.A. Prince. Dept. of Neurology and Neurological Sciences, Stanford University School of Medicine, Stanford, CA 94305.

Previous experiments have shown that the prolonged hyperpolarization which follows glutamate application to adult CA1 pyramidal neurons in hippocampal slices (postglutamate hyperpolarization or PGH) is due to activation of an electrogenic sodium pump (Thompson, S.M. & Prince, D.A., J. Neurophysiol., 56: 507, 1986). Na,K-ATPase activity is known to develop slowly postnatally (Haglund M.M. *et al*, Brain Res., 343: 198, 1985), however, little is known about its physiological role in immature neurons.

We assessed the development of Na pump activity in CA1 hippocampal neurons from slices of postnatal day (P) 7 to P39 rats. Spike amplitudes for neurons from P7-11, P21-25 and P35-39 age groups were 65.1±11.1, 83.7±7.6 and 84.3±8.0 mV, respectively; resting potentials were -71.9±8.0, -64.9±6.0 and -66.3±4.7 mV; and input resistances (R_N) were 50.2±20.1, 36.4±8.5 and 31.2±8.5 MΩ. Similar glutamate-induced depolarizations (GDs) could be elicited in all three age groups. The size (area) of PGH was directly proportional to the area of the preceding GD. A ratio of PGH area to GD area was calculated for each neuron and used to compare Na pump activities at different ages. The PGH ratios at ages of P21-25 and P35-39 were 0.82±0.42 (n=16) and 1.46±1.00 (n=5), respectively. However, in the P7-11 age group, PGH ratio was 0.26±0.13 (n=7), or significantly lower ($p<0.01$) when compared to the P21-25 value. Differences in membrane properties for these age groups do not explain the differences in PGH ratio. If anything, the higher R_N would tend to increase the sizes of PGHs in more immature neurons.

These results suggest that substantial Na,K-ATPase functional activity develops after birth. Lower levels of electrogenic Na pump activity at early stages of development could be one factor contributing to the increased susceptibility of immature hippocampus to ictal discharges associated with prolonged membrane depolarizations. Suppored by NIH grants NS02151 and NS06477 from the NINDS.

388.12

PATHWAYS OF SEIZURE AND INTERICTAL BURST PROPAGATION BETWEEN ENTORHINAL CORTEX AND HIPPOCAMPUS IN THE ZERO MAGNESIUM MODEL. A.C. Bragdon, H. Kojima and W.A. Wilson. VA Medical Center and SUNY HSC, Syracuse, NY, 13210; Akita U., Akita, Japan; VA and Duke University Medical Center, Durham, NC 27705.

Rat hippocampus (HC)/entorhinal cortex (EC) slices exhibit seizures and interictal bursts (IIBs) when bathed in medium containing no added magnesium (0-Mg medium). In general, the seizures arise first, then are suppressed by the IIBs. Here we describe the functional anatomy of the interaction between seizures and IIBs.

Recordings were made from EC and CA3 of HC/EC slices bathed in 0-Mg medium. In intact slices, seizures arose first in EC, but soon appeared synchronously in CA3. Seizures began in EC either spontaneously or triggered by a burst from CA3. Burst-by-burst analysis showed that EC bursts always led CA3 bursts except near the end of some seizures. Selective knife cuts revealed that seizures propagated from EC to CA3 both (1) with high efficiency via the perforant path, and (2) less efficiently via the temporoammonic path. IIBs arose in CA3, and evoked bursts responses in and suppressed seizures in EC. Knife cuts across subiculum prevented both these effects of IIBs.

These results show that seizures and IIBs can arise separately in EC and CA3, and that seizures can project from EC to CA3 over both perforant and temporoammonic pathways.

388.13

ELECTROGRAPHIC SEIZURES IN SLICES FROM OLDER RATS ARE INHIBITED BY PHYSIOLOGICAL LEVELS OF MAGNESIUM: RESTORATION OF SEIZURES IN LOW MAGNESIUM. W.W. Anderson and R.J. DeLorenzo. Dept. of Neurol., Med. Col. of Virginia, Richmond, VA 23298.

We have reported that electrographic seizures (EGSs) occur in hippocampal slices in physiological (0.9 mM Mg) medium when slices are from young (22-32 day old), but not older (>120 day old) rats (Stasheff et al. Science, 245:648, 1989). We report here that EGSs also occur in slices from older rats in Mg-free medium.

Hippocampal slices (625 uM) were prepared from 180-211 day old male Sprague-Dawley rats. Extracellular recording and stimulation were performed in stratum pyramidale and radiatum of CA3, respectively.

When slices were bathed in Mg-free medium + 2-5 uM of the $GABA_B$ agonist baclofen, EGSs either occurred spontaneously or following 1-3 stimulus pulses (N=7). These EGSs had the same tonic-clonic morphology and duration (mean = 59 sec) as the slices from young rats in Mg-free medium. However, the number of afterdischarge (AD) bursts per EGS (mean = 46) was only about half that of young rats. Addition of 50-200 uM of the NMDA antagonist D-2-amino-5-phosphonovalerate (APV) to Mg-free + baclofen medium substantially reduced, but did not block, AD bursting following 120 pulse stimulations (N=3).

When slices from older rats were bathed in 0.9 mM Mg + baclofen following EGS induction in Mg-free medium, 120 pulse stimulations produced only 0-5 AD bursts, indicating strong EGS inhibition (N=7).

These results suggest that the increased suppression of EGSs that occurs with aging is partially reversed by removing extracellular Mg. In slices from older rats, EGS inhibition could be due to Mg being more effective in stabilizing the membrane or decreasing transmitter release. Alternatively, NMDA antagonists do not block EGSs in slices from young rats in physiological medium (Stasheff et al.), indicating that non-NMDA-dependent mechanisms are sufficient to generate EGSs in these slices. In slices from older rats, the non-NMDA-dependent mechanisms alone may not be sufficient to generate EGSs in physiological medium, but may do so when augmented with NMDA-dependent mechanisms in Mg-free medium.

388.14

REDUCED SYNAPTIC INHIBITION UNDERLIES GENE-LINKED HIPPOCAMPAL NETWORK EXCITABILITY DEFECT IN THE EPILEPTIC MUTANT *TOTTERING* *S. A. Helekar* and J. L. Noebels*, Developmental Neurogenetics Laboratory, Section of Neurophysiology, Department of Neurology and Division of Neuroscience, Baylor College of Medicine, Houston, Texas 77030.

A gene-linked, voltage-dependent prolongation of network-driven paroxysmal depolarizing shifts (PDS) has been identified *in vitro* during 10 mM $[K^+]_o$-induced bursting in *tottering* (*tg/tg*) hippocampal CA3 pyramidal neurons (mean PDS durations: *tg/tg* 190±11, n=19; *+/+* 119±9, n=11). We have explored local circuit synaptic mechanisms in the CA3 region that might mediate this state-dependent inherited excitability defect. Blockade of NMDA receptors with bath-applied 40 µM (DL)APV produced approximately 30% reduction in PDS duration in both genotypes (*tg/tg* 135.0±13.0, n=5; *+/+* 84.0±11.1, n=5), but did not selectively correct the mutant phenotype, suggesting that increased synaptic excitation through NMDA receptors is unlikely to contribute to the altered network response. In contrast, blockade of $GABA_A$-receptors by 50 µM picrotoxin significantly prolonged (85%) the mean PDS duration in *+/+* neurons (217.8±35.9, n=4), but had no significant effect on the mutant PDS (173.0±20.5, n=5). These data support the hypothesis that the prolonged PDS in *tg* CA3 pyramidal neurons may reflect an underlying attenuation of $GABA_A$-receptor-mediated synaptic inhibition. Since intracellular recordings in *tg* CA3 cells under physiological 3 mM $[K^+]_o$ conditions clearly show spontaneous and evoked inhibitory synaptic potentials, this evidence raises the possibility that the *tg* mutation interacts with mechanisms regulating activity-dependent modifications in synaptic inhibition. (Supported by NIH and Pew Foundation (JLN)).

388.15

CHRONIC LESION OF MEDIAL SEPTAL NUCLEI INDUCES AN EPILEPTIFORM ACTIVITY IN RAT HIPPOCAMPAL SLICES. F.Berton*, R. Bianchi*, W.Francesconi*, B.Sidorov** & M.Brunelli. Dpt. Physiol. & Biochem. Univ. of Pisa,56100 Pisa, Italy - **Inst. of Higher Nervous Activity & Neurophysiol., Acad.of Sci. USSR, Moscow.

In the rat, hippocampal formation receives a well characterized septal projection from cholinergic and GABAergic neurons located in Medial Septal nuclei (MS) and vertical limb of Diagonal Band. The mechanisms underlying the effects of septo-hippocampal fibres degeneration on the excitability of the hippocampal neurons have not been carefully analyzed. Spontaneus and evoked multiple population spikes (MPSs) were recorded in CA3 and CA1 regions in slices isolated from animals sacrificed 1,2 or 4 weeks after electrolitical or chemical (local application of Ibotenic acid)lesion of MS. Spontaneous and evoked CA1-MPSs disappeared after cutting between CA3 and CA1 regions. In slices from unlesioned or sham-operated rats we never observed such an abnormal activity. In conclusion, septo-hippocampal fibres degeneration induces an epileptiform activity in CA3 spreading to CA1 region. Intracellular recordings from CA3 and CA1 neurons are in progress.

388.16

INFLUENCE OF ADENOSINE TRANSPORT AND ADENOSINE DEAMINASE INHIBITORS ON BICUCULLINE METHIODIDE-INDUCED SEIZURES IN RAT PREPIRIFORM CORTEX. G. Zhang, P. H. Franklin and T. F. Murray. College of Pharmacy, Oregon State University, Corvallis, OR 97331.

The results of our previous studies suggest that an adenosine receptor population in rat prepiriform cortex (PPC) plays an important role in the suppression of bicuculline methiodide (BMI) and kainic acid-induced seizures (Franklin, P.H. *et al.*, *J. Pharmacol. Exp. Ther.*, 251:1229,1989; Zhang, G. *et al.*, *Neurosci. Lett.*, in press). In the present study we evaluated the manipulation of endogenous adenosine in this brain area as a strategy to effect seizure suppression. All compounds used in these experiments were unilaterally microinjected into the PPC. It was found that the co-administration of a subconvulsant dose (1.6 nmol) of the adenosine antagonist 8-*p*-(sulfophenyl)theophylline (8pSPT) with BMI (30 pmol) markedly enhanced the severity of BMI seizures. Dilazep, an adenosine transport inhibitor, provided a potent protection against BMI seizures with an ED_{50} value of 5.6±1.6 nmol/rat. Together, the proconvulsant effect of 8pSPT and anticonvulsant action of dilazep indicate that endogenous adenosine in the PPC exerts an inhibitory tone which affects seizure susceptibility. Furthermore, we found that exogenous adenosine also afforded a dose-dependent suppression of BMI-induced seizures (ED_{50}=48.1±8.4 nmol/rat) in the PPC, and these anticonvulsant effects of adenosine were significantly potentiated by the adenosine transport blockers dilazep (0.5 nmol) and nitrobenzylthioinosine phosphate (20 nmol). Similarly an adenosine deaminase inhibitor 2'-deoxycoformycin (11.2 nmol) produced 2.9 fold potentiation of the anticonvulsant effect of adenosine. These findings therefore imply that adenosine is a major modulator of neuronal activity in the PPC and the inhibition of the adenosine transport system and/or adenosine deaminase influences extracellular adenosine levels in this paleocortical brain area. (Supported by USPHS Grant NS23227).

388.17

IN VIVO AND IN VITRO ASSESSMENT OF NEUROTRANSMITTER AMINO ACID FUNCTION IN TETANUS TOXIN INDUCED CHRONIC SEIZURE FOCI IN RAT HIPPOCAMPUS. C.M. Forchetti, B. Leheta*, and D. Garant*. Dept Neurological Sciences, Rush Medical College, Chicago IL 60612.

Tetanus toxin or vehicle was injected unilaterally into dorsal hippocampus of rats with chronic depth electrode/guide cannula implants. After injection, EEG and focal in vivo micro-dialysis were performed in the unanesthetized rats, either before seizure onset, during the peak of limbic seizures, or after seizure remission. Enriched synaptosomal fractions were prepared from hippocampi dissected from these rats, and amino acid concentrations were assayed by reverse-phase HPLC.

As expected, in tetanus toxin seizure foci in vivo, K^+-induced GABA release appeared decreased, however synaptosomal GABA content was remarkably consistent through the seizure course and between toxin and vehicle groups. This suggests that tetanus toxin inhibits GABA release, but neither this effect nor the subsequent seizures alter nerve terminal GABA concentration.

388.18

OUABAIN-BINDING SITES AND mRNAs OF Na,K-ATPases ARE DECREASED IN HIPPOCAMPAL AREA CA1 OF RAT BRAIN AFTER KAINATE LESIONS OF CA3. A.A. Maki, J. E. Franck and W.L. Stahl . VA Medical Center and Univ. Washington Sch. Med., Seattle, WA 98108.

Administration of kainate to rats causes loss of neurons of the hippocampal CA3 region and leads to a hyperexcitable state in CA1 pyramidal neurons. We hypothesize that changes in ion homeostasis, especially due to decreased expression of the Na,K-ATPase, may contribute to the mod-ified properties of CA1 neurons. We used ouabain binding to measure the number of Na,K-ATPase sites in CA1 AND CA3 areas (J. Neuro-chem. 52: 193-200, 1989) and in situ hybridization (Neurosci. Res. Commun. 5: 155-162, 1989) to measure expression of mRNA for Na,K-ATPase subunits. Both high and low affinity [^{3}H]ouabain binding sites were found in control and lesioned hippocampus with K_d values of 60-77 nM(high) and 960-1382 nM (low). No differences were found in K_d values between control and lesioned animals. Numbers of low affinity binding sites differed only in CA3 where nearly complete destruction of pyramidal cells was observed. High affinity sites were decreased in CA1 as follows: stratum oriens, 20%; stratum radiatum, 25%; pyramidal cell layer, 33%. In addition significant loss of mRNA encoding for α2 and α3 isoforms of the catalytic subunit of the Na,K-ATPase was found in CA1 pyramidal cells. Since Na,K-ATPases containing α2 and α3 are known to bind ouabain with high affinity, the in situ hybridization studies support the ouabain-binding results. We conclude that the number of Na,K-ATPase molecules with high affinity for ouabain in CA1 pyramidal cells is significantly reduced and may lead to modified ion homeostasis in this model of epilepsy. (Supported by NS 20482 and the VA.)

388.19

MODIFICATION OF HYPEREXCITABILITY IN THE SUBCORTICALLY DENERVATED HIPPOCAMPUS BY SEPTAL GRAFTS.

M. Hsu, Z. Horvath*, F. H. Gage, C. Slamka* and G. Buzsaki.
Department of Neurosciences, UCSD, La Jolla, CA 92093.

Suspension grafts of basal forebrain cholinergic neurons were implanted into the hippocampus 3 months after fimbria-fornix (FF) transection and the emergence of hyperexcitability (Neurosci. 28:527,1989) was tested 9 months after grafting. The threshold for evoking afterdischarges was significantly elevated in the grafted rats relative to FF-only animals. The correlation between perforant path evoked extracellular PSP and population spike was positive in unoperated control (UC) rats and grafted rats but negative in FF-only rats. The behavior-dependence (walk vs. still) of pop-spike amplitude was largest in the FF group, and smallest in the UC animals with the grafted rats intermediate. Behavior-dependence of PSP was similarly larger in FF and grafted groups relative to UC rats. Long-term potentiation (LTP) of the dentate population spike was impaired in the FF-only rats; grafting did not result in significant improvement of LTP. High frequency (18-32/sec) power in the hilus was significantly larger during walking than during immobility in all groups, and behavioral modulation was significantly larger in the grafted animals and FF rats than in the UC group. We conclude that delayed grafting of cholinergic cells is capable of modifying the already established hyperexcitability patterns of the denervated hippocampus, although not all changes are restorative.

389.1

ABNORMAL LYSOSOMAL HYDROLASE DISTRIBUTIONS IN ALZHEIMER BRAIN. A. M. Cataldo*, A. Pope and R. A. Nixon. McLean Hospital, Harvard Medical School, Belmont, MA 02178

Lysosomal proteinases in enzymatically active form are abnormally present extracellularly in classical senile plaques (SP) (Cataldo and Nixon, PNAS, 1990). We have proposed that degenerating cathepsin-laden neurons and their processes are the principal source of these enzymes in SP and that the unregulated action of lysosomal hydrolases leads to abnormal metabolism of the amyloid precursor protein (APP). To further establish this idea, we localized other lysosomal hydrolases in Alzheimer brain. Sections of neocortex from control and AD brains were analyzed by immunocytochemistry with polyclonal antisera to the non-protease enzymes, α-galactosidase (GAL), α-hexosaminidase A (HEX A), and α-glucosidase (GLU) and by enzyme cytochemistry for aryl sulfatase and acid phosphatase activities. Senile plaques were also identified histologically using Bielschowsky silver stain and thioflavin S histofluorescence. HEX A, GAL and GLU were present in neurons of control and AD brains and were abundant in senile plaques of AD brain. In SP, these hydrolases were localized by immunoelectron microscopy to extracellular membrane-limited profiles. Neurofibrillary tangles were also intensely stained with GAL antiserum. The activities of aryl sulfatase and acid phosphatase were detected in many SP even though activities in neurons were below the sensitivity limit of the method. The abnormal localization of various lysosomal hydrolases with extracellular β-amyloid deposits supports a model involving neuronal degeneration and persistence of the lysosomal compartments which are capable of unregulated proteolysis including possibly abnormal breakdown of APP. The aberrant trafficking and release of membrane-degrading hydrolases may be one mechanism to expose the intra-membrane A4 cleavage site.

389.2

COMPLEMENT PROTEINS ARE ASSOCIATED WITH MEMBRANE ATTACK IN ALZHEIMER DISEASE. P. L. McGeer, H. Akiyama, T. Kawamata*, E. G. McGeer. Kinsmen Lab. of Neurol. Res., Dept of Psychiatry, Univ. of British Columbia, Vancouver, B.C., Canada, V6T 1W5.

The pattern of Alzheimer tissue staining by antibodies to complement and related proteins indicates that active cell lysis as well as tissue opsonization is taking place. Extracellular debris in the form of amyloid deposits and ghost tangles are strongly stained by antibodies to the complement proteins C1q, C3d and C4d. These proteins are associated with the opsonizing function of complement. Antibodies to vitronectin, a serum protein which binds to the C5b-9 membrane attack complex (MAC), also stain these structures. Despite the presence of this inhibitor of MAC insertion into cell membranes, diseased neuronal elements, which are identifiable by antibodies to ubiquitin and Tau-2, are stained by an antibody to a neoantigenic site on the C5b-9 complex. Many of these same tangled neurons and dystrophic neurites are also stained by an antibody to the membrane inhibitor of reactive lysis (MIRL, JCI 84:7-17, 1987, courtesy of Dr. C.J. Parker, Univ. of Utah). MIRL restricts the susceptibility of human erythrocytes to complement-mediated hemolysis induced by activated cobra venom. This inhibitor of bystander lysis is a phosphatidylinositol-linked 18 kd protein which inhibits assembly of the membrane attack complex at the level of C7 and C8 incorporation. MIRL might be induced in neurons subject to bystander lysis by the C5b-9 complex. These data suggest that inhibition of complement activated cell lysis might reduce the rate of cellular degeneration in Alzheimer disease.

389.3

AD PATHOLOGY: IN VIVO QUANTITATION BY MRI? J.W. Ashford, A. Rashid*, D. Lawrance*, M. Calache*, J. Bice*, G. Evans* and D. Anderson*. SIU Sch. of Med. Springfield, IL 62794.

The critical diagnostic issue for Alzheimer disease (AD) is regional quantitation of pathology in vivo. Magnetic resonance imaging (MRI), capable of measuring a variety of cerebral characteristics with considerable dynamic range, might achieve this quantitation. For example, senile plaques (60 μ dia., about 10/mm^2 at diagnostic threshold = 3% of tissue volume) might have a property which can be assayed. Spin density images (TR=2 s; TE=40 ms, balancing T1/T2 properties, not distinguishing gray/white matter/CSF) highlight several types of pathology. Using this protocol, statistical enhancement (fig. 1) of coronal images (fig. 2) showed a pattern of intensities in 6 probable AD patients suggestive of the distribution of AD pathology (data shown from autopsy-confirmed AD patient).

Fig. 1

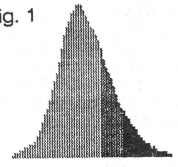
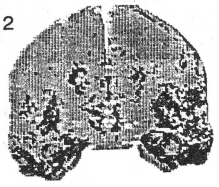


Fig. 2



389.4

MORPHOLOGICAL ALTERATIONS OF NEUROPEPTIDES IN THE AMYGDALA IN ALZHEIMER'S DISEASE (AD). W.C.Benzing, E.J. Mufson§, and D.M. Armstrong. FGIN, Georgetown University, Washington, D.C. 20007; §IBR, Sun City, AZ 85372.

We used immunocytochemistry in order to examine the morphologic features of three neuropeptides (somatostatin (SOM), substance P (Sub P), and neurotensin (NT)) and of choline acetyltransferase (ChAT) in the amygdala of patients with AD and in 2 groups of controls. One control group (normal) had few if any plaques, while the other group (high "N") had numerous plaques. The topography of the pathologic lesions was determined using Thioflavin-S. In AD patients and the high "N" controls, immunolabeling for ChAT and NT was reduced in density relative to normal controls, while Sub P and SOM labeling was reduced only mildly if at all. The reductions in NT and ChAT immunoreactivity were more pronounced in the AD cases than in the high "N" cases. Despite these differential reductions in density, neuritic fibers of all four neurotransmitters showed similar morphologic alterations in both the AD cases and the high "N" cases but these were rarely observed in the normal controls. These alterations were characterized by gross varicose swellings which were often associated with plaques and were most prevalent in areas of high plaque density. Most importantly, in the areas of highest plaque formation in the AD cases (which coincided with the areas of plaque formation in the high "N" cases), ChAT and NT processes were nearly or completely absent and were thus rarely found within plaques, while in these same areas in the high "N" controls, ChAT and NT were present in reduced amounts and had abnormal fibers that were found within plaques. Abnormal SOM and Sub P fibers were present within plaques in these areas in both the AD and the high "N" cases. The observation that all four neurotransmitters displayed similar morphologic irregularities suggests a common pathological process. The reductions in ChAT and NT suggest that these transmitter systems may be more susceptible to the pathologic changes.

389.5

THE NUCLEUS BASALIS SHOWS LITTLE CELL LOSS OR OTHER PATHOLOGICAL CHANGE IN MILD ALZHEIMER'S DISEASE. J.L. Price, J.C. Morris, P. Davis & D.L. White. Depts. of Anat./Neurobiol. and Neurology, Washington Univ. Sch. Med., St. Louis, MO 63110

Previous studies have reported that the nucleus basalis of Meynert (NBM) undergoes substantial cell loss in Alzheimer's disease, associated with loss of cholinergic markers in the cortex. It has been suggested that this might be partially responsible for the cognitive decline associated with the disease. As part of an ongoing study of the relation between normal aging and Alzheimer's disease, we have determined the density of neurons, tangles, and cells immunoreactive for the Alz-50 antibody and an antibody against paired helical filaments (donated by Drs. Peter Davies and Dennis Selkoe) in the cell clusters of the NBM. The cases studied were evaluated in the Alzheimer's Disease Research Center of Washington University and assigned a Clinical Dementia Rating (non-demented: CDR=0; very mildly demented to severely demented: CDR=0.5 to 3).

The density of large neurons was determined at three levels of the NBM. Four mildly demented cases (CDR=0.5 to 1) showed no loss of cells when compared to three non-demented cases (ca. 65 cells/mm^2). However, three more severely demented cases (CDR=2 to 3) had much lower cell density (ca. 20 cells/mm^2). The density of tangles and immunoreactive cells in the NBM of five mildly demented cases was slightly above that seen in non-demented elderly cases (average tangle density=2/mm^2 vs 0.4/mm^2). The density of these markers was greater in the severely demented cases (6.1/mm^2), but is much less than in the hippocampal formation or neocortex. These observations indicate that cellular damage in the NBM is not a substantial feature of the early stages of Alzheimer's disease; it might contribute to the cognitive loss in later stages of the disease, but in such cases there is also substantial damage to the hippocampus and neocortex.

Supported by NIH grants AGO3991 and AGO5681.

389.6

BASAL FOREBRAIN PROJECTIONS TO THE FRONTAL POLE OF THE THALAMIC RETICULAR NUCLEUS (MEDIODORSAL NUCLEUS AND FRONTAL CORTEX TERRITORY) IN THE OLD-WORLD MONKEY, AND THEIR DISRUPTION IN ALZHEIMER'S DISEASE. W.G. Tourtellotte, I.A. Ilinsky and G.W. Van Hoesen. Depts. of Anatomy and Neurology, Univ. of Iowa, Iowa City, IA 52242.

The thalamic reticular nucleus (Rt) forms a narrow band of largely GABAergic neurons around the margins of the thalamus. It receives thalamocortical, corticothalamic, and cholinergic fibers from the basal forebrain (BF) and the pontomesencephalic reticular formation (PMRF). Physiological studies indicate that the BF and PMRF can disinhibit Rt neurons and facilitate thalamic transmission. Immunocytochemistry with Alz-50 and tau antibodies in Alzheimer's disease (AD; N=15) but not control brains (N=8) reveals terminal-like immunostaining in the rostral pole of Rt. Nerve growth factor receptor (NGFr) immunocytochemistry and NADPH-diaphorase (NADPHd) histochemistry were used to assess the integrity of cholinergic neurons in the septal-BF complex and PMRF, respectively, in some of the AD and normal brains. There was a consistent depletion of neurons and numerous thioflavin S, Alz-50 and tau positive neurons in BF but not in PMRF. Old-World monkeys received iontophoretic injections with either PHAL or WGA-HRP in the BF and Rt. HRP injections confined to the rostral pole of Rt demonstrated retrograde transport to the mediodorsal nucleus (MD), medial prefrontal and orbitofrontal cortices, BF and PMRF. In the BF, retrogradely-labeled neurons were located in intermediate and caudal locations. Iontophoretic injections with PHAL confined to the pole of Rt revealed dense projections to MD. The data suggest that corticothalamic and thalamocortical fibers interconnecting MD and frontal cortex synapse in the pole of Rt. They also demonstrate that the Rt pole receives cholinergic projections from the BF and the PMRF. Since neither the neurons in the PMRF nor MD appeared consistently involved in AD, the changes observed in the Rt may well arise from Alz-50 and tau positive neurons in the posterior and intermediate sectors of the BF. This may reflect a disruption of modulatory influences by the BF on MD-frontal lobe interactions in AD. (Supported by: 5T32 GM0733, NS 14944, PO NS 19632.)

389.7

LN-1 IMMUNOREACTIVITY IS SPECIFIC TO ASTROCYTES IN CORTEX OF NORMAL AGED AND ALZHEIMER'S DISEASE (AD) BRAIN TISSUE. G. Moon*, S.D. Styren, W.H. Civin, and J. Rogers, L. J. Roberts Center, Institute for Biogerontology Research, Sun City, AZ 85351.

LN-1 is a monoclonal (IgM) antibody specific for an uncharacterized cell membrane antigen present on B lymphocytes but not T lymphocytes or histocytes. A previous report suggested that LN-1 is a specific marker for CNS microglia. We find, however, that this antibody specifically labels CNS astrocytes only. Moreover, astrocytes with reactive morphology--as found particularly in AD brain tissue--exhibit increased expression of the LN-1 antigen.

Frontal cortex samples from 9 AD and 9 aged-matched neurologically normal controls were obtained at autopsy (mean autolysis time of 4 ± 2 hours), cut into 1 cm blocks, fixed for 24 hours in 4% buffered paraformaldehyde, vibratome sectioned at 50 μm, and processed for immunohistochemistry. Additional material for immunohistochemistry was paraffin embedded after 10% or 20% formalin fixation for various times.

Intense LN-1 immunoreactivity is observed on highly reactive astrocytes near the border of cortical layers I and II. Both the intensity of staining and the number of labelled cells are elevated in AD. LN-1 immunoreactivity in the other cortical layers occurs on fewer astrocytes, the labelling is weaker, and the morphology is considerably less reactive. LN-1 positive astrocytes, typically with nonreactive morphology, occur diffusely in the white matter. The specificity of the LN-1 antibody for astrocytes (and only astrocytes) is confirmed by morphologic criteria and by double-labelling with the astrocyte specific marker GFAP.

Increased expression of the immune system associated antigen LN-1 by cortical astrocytes in AD is consistent with demonstrations by our laboratory and others that the pathogenesis of AD includes a chronic inflammatory component.

[Supported by NIA AGO7367 to JR]

389.8

MORPHOLOGIC EVIDENCE OF A DYNAMIC RELATIONSHIP BETWEEN MICROGLIA AND SENILE PLAQUE AMYLOID IN ALZHEIMER DISEASE (AD). L.S. Perlmutter, M.A. Myers*, E. Barron*, and H.C. Chui. USC School of Medicine, Dept Neurology, RMR-407, Los Angeles, CA 90033.

Amyloid and mononuclear phagocytic cells are intimately associated in several amyloidoses. In AD, amyloid/microglia associations have been seen with light (LM) and electron microscopy (EM). With EM, bundles of amyloid from compact cores were found interdigitated with microglial cell membrane and within intracellular membraneous cisterna. These cells were morphologically characterized as resting microglia - suggesting a secretory rather than phagocytic role.

The present study examined the relationship between microglial morphology and the pattern of amyloid accumulations in AD. Microglial cells were labeled with the lectin *Ricinus communis* agglutinin-1 for LM and EM. With LM, dense amyloid cores (stained with thioflavine S) were always tightly encircled by clusters of highly ramified microglia. Larger, diffuse amyloid plaques were associated with globular-shaped microglia with few if any processes. With EM, cytoplasm from labeled resting microglia were found interdigitated with amyloid bundles; intracellular bundles were also seen. Round, dense body-filled microglia were scattered around diffuse amyloid accumulations.

These data suggest a complex and dynamic pathogenetic relationship, which may vary with the microglia's functional state: ramified microglia may secrete while rounded microglia may phagocytose amyloid. Thus, compact rather than diffuse amyloid accumulations may represent an earlier form of senile plaques. (AG07127, AG05142, AG07624)

389.9

THE BRAINSTEM IN ALZHEIMER'S DISEASE (AD). T. Doucette*, T. Duong and A. B. Scheibel. UCLA Anatomy Department, Brain Research Institute and Mental Retardation Research Center, L.A., CA 90024.

The immunohistochemical labeling by antisera to amyloid P component (AP-C) has been compared to that by antisera raised against microtubule-associated protein tau (MAP-tau) and paired helical filaments (PHF) in the pons of AD patients. Brain tissue was fresh fixed in 4% paraformaldehyde by immersion, cut on a cryostat (40 μm section thickness) and processed by the peroxidase-antiperoxidase method. The principal neuropathological lesions observed were neurofibrillary tangles (NFT) in the locus ceruleus and raphé regions. The antiserum to MAP-tau labeled intracellular NFT and neuropil neurites in areas without NFT. A diffuse MAP-tau labeling without intracellular presence of NFT was also observed in some neurons. MAP-tau antiserum may label neurons which potentially develop NFT. The antiserum to PHF labeled extracellular NFT and surrounding neurites. It also revealed neuropil neurites but to a much smaller extent than the MAP-tau antiserum. AP-C antisera labeled mostly extracellular NFT although a few intracellular NFT were also observed. Neurites in the immediate area around extracellular NFT were labeled by AP-C antisera but neuropil neurites in areas lacking NFT were not. Thus the labeling of neuropathological elements in the pons by AP-C antisera is qualitatively more similar to that of PHF antiserum. We suggest that in NFT formation, MAP-tau antiserum is able to label neurons which potentially develop NFT, whereas AP-C antisera label only neurons in which PHF are present in the soma. We conclude that **AP-C may not be a causative agent in the formation of NFT but only binds to NFT after the PHF are present.**

389.10

IMMUNOHISTOCHEMICAL ANALYSIS OF THE NUCLEUS BASALIS OF MEYNERT (nbM) IN ALZHEIMER'S DISEASE (AD). R.W. Jacobs, T. Duong, and A.B. Scheibel. Depts. of Anatomy and Psychiatry and the Brain Research Institute, UCLA, CA 90024.

The histopathology of the rostral cholinergic nuclei (nbM, diagonal band of Broca, and medial septum) in AD has been compared to the neocortex, amygdala, and hippocampus. Brain tissue from AD and aged controls were processed for Nissl, thioflavin-S, and acetylcholinesterase (ACHE); and immunohistochemically labeled for paired helical filaments (PHF), MAP-tau, ß-amyloid, amyloid P-component (AP-C), choline-acetyl transferase (ChAT), and glial fibrillary acid protein (GFAP). Numerous neurofibrillary tangles (NFT) and plaques were noted in the AD neocortex, amygdala, and hippocampus by thioflavin-S, PHF, MAP-tau, and AP-C. The cores and neurites of senile plaques were positive for ß-amyloid. GFAP demonstrated large fibrous astrocytes associated with plaque formation. The cholinergic nuclei displayed neurons in various stages of degeneration, many hypertrophied astrocytes, a lack of ß-amyloid except in arteriolar walls, and in some regions end-stage gliosis without the presence of neuronal elements including residual NFT. A significant proportion of the remaining ACHE and ChAT-positive neurons contained globular NFT. These were demonstrated by thioflavin-S, PHF, and MAP-tau but not by AP-C which identified only a few lightly labeled NFT. We suggest the existence of two types of NFT: Those found in the cholinergic nuclei which are largely AP-C negative and seem to disappear with neuronal loss; and those from other brain regions which are AP-C positive and tend to accumulate. **The finding of atypical, non-accumulating NFT and a paucity of senile plaques and ß-amyloid deposition may point to a pathogenic expression unique to the rostral cholinergic nuclei.**

389.11

CHOLINERGIC ALTERATIONS IN CORTICAL BIOPSY TISSUE IN ALZHEIMER'S DISEASE. S.T. De Kosky , R. Harbaugh*, S.W. Scheff, Schmitt, F.A.* and the intraventricular bethanechol study group. Alzheimer's Disease Res. Ctr. & Dept. of Psychiatry, Univ. of Pittsburgh, Suite 400, 3600 Forbes Ave., Pittsburgh, PA 15213, Dartmouth Univ. and Univ. Kentucky Med. Ctrs. & Sanders-Brown Center on Aging, Lexington, KY.

Neurochemical assessments were performed on biopsy samples taken from the right frontal lobe of patients diagnosed with Alzheimer's disease (AD), prior to the implantation of a ventricular catheter and pump assembly for the infusion of bethanechol as an experimental therapy. Samples were obtained from 6 different medical centers. The pathologically confirmed AD cases (n = 26) were compared to a group of samples from normal age-matched autopsied controls (n = 21) and age-matched autopsied AD brains (n = 11). All samples were assayed for choline acetyltransferase (ChAT), acetylcholinesterase (AChE), binding to ^{3}H-quinuclidinyl benzilate (QNB) as an index of total muscarinic cholinergic binding and ^{3}H-pirenzepine (PZ) binding as an index of M1 receptor subtype binding. Mean levels of ChAT activity were decreased to 36% of age-matched controls, and the loss of activity correlated significantly with the mini-mental state examination, an index of global cognitive function. ChAT activity in autopsied AD cortex was further decreased compared to controls, indicating continuous further decline through the course of the disease. AChE followed a similar, less dramatic decline. No differences were found in QNB binding among the groups but PZ binding was significantly decreased in autopsied AD cases compared to biopsy cases or autopsied controls.

389.12

CT WHITE MATTER LUCENCIES (WML) IN AGING AND ALZHEIMER'S DISEASE (AD) DO NOT CORRELATE WITH GLOBAL COGNITIVE IMPAIRMENT. A. Sudilovsky, M.J. de Leon*, G.S. Smith, A.E. George*, S. Repetti and J. Ciaravino*. The Squibb Inst. for Med. Res., Princeton, NJ and NYU Med. Cntr., NY, NY.

We conducted a multicenter investigation of WML (decreased CT density) in normal elderly volunteers (N=301; Mini-Mental State Examination score (MMSE) above 27), AD patients (N=273; MMSE: 12 to 23), and patients with minimal cognitive deficit (MCD; N=59; MMSE: 24 to 27). Criteria for admission included: age 50 to 85 years, ischemia score below 3, blood pressure below 160/95, and no use of antihypertensives in prior 3 months. All participants received non contrast GE 8800 or GE 9800 CT scans (base to vertex of the brain)yielding slices 10 mm in thickness. All slices in each of 3 bilateral areas in each case (frontal lobe; parietal/peritrigonal area; and diffuse involvement) were rated on a 7 point scale. Ventricular and sulcal dilatation were similarly rated. WML were found in 21% of normal controls, 29% of AD patients, and 25% of MCD patients. In each group, patients with MWL were older (p 0.05). Controlling for age differences indicated greater ventricular enlargement and sulcal dilatation accompanying WML. This was most prominent in normal controls and mildly affected patients. Further atrophy independent of WML was observed in more severely affected patients.

Our results demonstrate the increasing prevalence of WML with age and their association with atrophy in aging and early AD. No relationship was found between MMSE and WML severity.

389.13

POSSIBLE NEURONAL PARTICIPATION REVEALED BY PKC(B2) IMMUNOREACTIVITY IN ALZHEIMER DISEASE (AD) PLAQUES. E.Masliah, R.D.Terry*, L.Hansen*, M.Mallory*, G.Cole, T.Saitoh. Dept. of Neurosciences, U.C.S.D., La Jolla, CA 92093.

A previous report suggested involvement of PKC in AD plaques. We have been studying the spatial relationships between the anti-PKC(B2) labeled component and various cellular populations; i.e., neurons, microglia and astrocytes in this lesion. AD preparations were 40u vibratome sections from the midfrontal cortex and hippocampus. The sections were double immunolabeled with: a) anti-PKC(B2)/SMI-31(neuronal marker), b)anti-PKC(B2)/anti-synaptophysin (presynaptic terminal marker), c) anti-PKC(B2)/RCA-1 (microglial marker), and d) anti-PKC(B2)/anti-GFAP (astrocytic marker).Additional sections were double-labeled with anti-amyloid B/A4. The sections were then incubated with a combination of secondary antibodies tagged with FITC and Texas Red for study with the Bio-Rad MRC 600 laser confocal system. Observations of serial optical sections suggest that the PKC(B2) immunoreacting components of the plaques are actually SMI-31 positive, short, sprout-like processes extending from neurons in the area of the diffuse plaque. The cellular processes of astrocytes and microglia were associated with amyloid and neuronal perikarya in the classical plaque. The PKC(B2) immunostained processes in the classical plaques seemed to be closely apposed to amyloid fibrils.

THURSDAY AM — SYMPOSIA

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SYMPOSIUM. GENETICALLY MODIFIED CELLS: DEVELOPMENT AND APPLICATION FOR THE NEUROSCIENCES. B.H. Wainer, The Univ. of Chicago (Chairperson); J.R. Sanes, Washington Univ., St. Louis; D.J. Anderson*, Cal. Tech.; M.R. Capecchi*, Univ. of Utah; F.H. Gage, Univ Cal., San Diego.

The participants in this symposium will present recent approaches employing genetically modified cells to study areas of specific interest to the neurosciences: cell lineage and development, trophic interactions, and intercellular signalling. Dr. Sanes will describe the application of retrovirus-mediated gene transfer into neural cells *in vivo* to trace neural cell lineage in the embryonic mouse and chick nervous systems. Dr. Anderson will describe the isolation of sympathoadrenal progenitor cells by fluorescence activated cell sorting using specific cell surface monoclonal antibodies, and the immortalization of these progenitors by retroviral-mediated gene transduction to study sympathoadrenal lineages that generate the major catecholamine-containing derivatives of the neural crest. Dr. Capecchi will discuss the use of gene targeting for the introduction of specific mutations into mouse embryonal stem cells *in vitro* which can then be employed to generate transgenic animals for evaluation of the consequences of such mutations *in vivo*. Dr. Wainer will discuss the use of somatic cell fusion approaches to generate clonal cell lines from postmitotic embryonic and young adult neurons to study trophic interactions as well as other neural processes. Dr. Gage will describe the generation of non-neuronal cell lines transfected with cDNA for either trophic factors or enzymes relevant to specific neurotransmitter systems, the synthesis and secretion of active transgene products by these cells, and the grafting of such cell lines into adults brains to induce long-term functional effects.

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SYMPOSIUM. DIFFERENTIAL PROCESSING OF VISCERAL AND SOMATIC INPUT IN THE CENTRAL NERVOUS SYSTEM. R.D. Foreman, Univ. of Okla. HSC (Chairperson); W.C. deGroat, Univ. of Pittsburgh; F. Cervero, Univ. of Bristol; G.F. Gebhart, Univ. of Iowa; R.W. Blair, Univ. of Okla. HSC; W.D. Willis, Univ. of Texas at Galveston.

The objective is to compare and contrast the similarities and differences of visceral and somatic processing in the peripheral and central nervous system. Dr. deGroat will discuss the similarities and differences in the neurochemistry and anatomy of afferent pathways to the lumbosacral spinal cord. He also will discuss morphological plasticity in visceral afferent pathways. Dr. Cervero will discuss the functional changes in visceral nociceptors that follow sensitization and the plasticity of somatic and visceral spinal neurons following prolonged noxious stimulation. Dr. Gebhart will present studies showing spinal and supraspinal responses of cells distention of the normal and inflamed colon. Dr. Foreman will compare and contrast the organization of spinothalamic tract cells that receive cutaneous, muscle and visceral input and project to the oral and caudal regions of the ventral posterior lateral thalamic nucleus. Dr. Blair will summarize how different regions of the medullary reticular formation integrate visceral, primarily cardiac, and somatic information. Dr. Willis will discuss how thalamocortical structures integrate visceral and somatic input. He will conclude with his perspective concerning future directions that need to be pursued to provide the most effective development of this field.

HYPOTHALAMIC-PITUITARY-GONADAL REGULATION II

393.1

PRO-OPIOMELANOCORTIN (POMC) NEURONS IN THE RAT MEDIOBASAL HYPOTHALAMUS (MBH) PROJECTING TO THE MEDIAL PREOPTIC AREA (MPO) ARE SYNAPTIC TARGETS OF GABAERGIC TERMINALS. F. Naftolin[1] M. Shanabrough*[1] and C. Leranth[1 2]. Dept. of Obstetrics and Gynecology[1] and Section of Neuroanatomy[2], Yale Univ. Sch. of Med., New Haven, CT. 06510

We have previously shown that POMC peptide-containing neurons provide a direct link between the arcuate nucleus (AN) and MPO LHRH neurons. The AN contains GABAergic interneurons which could control the AN POMC neurons. To test the hypothesis that pacemaker impulses from such GABAergic MBH integrator unit neurons are projected to MPO LHRH neurons via the POMC cells, the connections between these cells were studied using a combination of retrograde tracing and double immunostaining procedures. Experimental: horseradish peroxidase (HRP) was stereotaxically injected in the MPO of adult female rats. On the next day, animals were colchicine treated (80μg in 20μl saline, injected into the lateral ventricle) and then sacrificed 24 hrs later by transcardial perfusion of fixative. The retrogradely transported HRP was visualized using the glucose oxidase reaction on vibratome sections of the MBH. In addition, we immunostained for β-endorphin and GAD by a double immunostaining procedure using two contrasting electron dense markers, IgG conjugated 5nm gold for β-endorphin-containing cells and immunoperoxidase reaction for GAD-containing neurons. Results: EM of triple labeled sections showed a population of the β-endorphin immunoreactive neurons located mostly in the lateral part of the arcuate nucleus, to contain retrogradely transported HRP granules from the MPO and are innervated by GAD immunoreactive boutons. Conclusions: GABAergic neurons innervate the POMC tract from the AN to the MPO LHRH neurons. Since these are likely inhibitory connections and are estrogen sensitive (Wallis and Luttge, '82), they may in part explain estrogen's control inhibition of gonadotrophins. Supported by NIH Grant HD23830

393.2

EVIDENCE THAT NEUROPEPTIDE Y (NPY) PLAYS A ROLE IN THE PUBERTAL INCREASE IN LHRH RELEASE IN THE FEMALE MONKEY. A. C. Gore & E. Terasawa. Neurosci. Training Prog. & Wisconsin Reg. Primate Res. Ctr., Univ. Wisconsin, Madison, WI 53715

An increase in pulsatile LHRH release from the hypothalamus is critical for the initiation of puberty. However, the neural mechanism controlling this pubertal increase in LHRH is unknown. Since we found that NPY plays a role in the control of pulsatile LHRH release in adult monkeys, we examined whether NPY contributes to the increase in LHRH release that occurs during puberty using push-pull perfusion of the stalk-median eminence (S-ME). In the 1st experiment, endogenous NPY release in the S-ME of 10 prepubertal (12-20 mo) and 11 midpubertal (31-46 mo) monkeys was measured by RIA. No developmental changes in NPY release were observed: mean NPY release was 204±44 and 234±80 pg/ml for pre- and midpubertal monkeys, respectively. Thus, in the 2nd experiment we tested whether the responsiveness of the LHRH neuronal system to exogenous NPY changes during puberty. NPY (10^{-8} & 10^{-6}M) or vehicle was infused to the ME for 10 min through the push cannula and LHRH levels in samples collected at 10 min intervals were measured by RIA. In prepubertal monkeys (n=5), mean LHRH release was 1.3±0.2 pg/ml and was not affected by infusion of either NPY or vehicle. In contrast, in midpubertal monkeys (n=5) NPY infusion significantly stimulated LHRH release ($p<0.01$) from 4.3±1.4 to 6.5±1.9 pg/ml after 10^{-8}M NPY and from 3.6±1.1 to 6.1±1.4 pg/ml after 10^{-6}M NPY. Vehicle infusion did not affect LHRH release. These results suggest that while NPY levels do not change, an increase in the responsiveness of the LHRH neuronal system to NPY occurs during the pubertal process. Therefore, we conclude that the pubertal increase in pulsatile LHRH release is, in part, due to an increased sensitivity to NPY. (HD11355, RR00167 & GM07507).

393.3

NEUROPEPTIDE Y (NPY) ENHANCES LHRH-STIMULATED LH SURGES IN PENTOBARBITAL-BLOCKED PROESTROUS RATS. A. C. Bauer-Dantoin, J.K. McDonald[1], and J.E. Levine. Dept. Neurobiol. & Physiol., Northwestern Univ., Evanston, IL 60208 & [1]Dept. Anat., Emory Univ., Atlanta, GA 30322.

NPY may enhance the secretory response of gonadotropes to LHRH during initiation of the LH surge. To test this hypothesis, effects of NPY on LHRH-stimulated LH secretion were assessed in pentobarbital (PB)-anesthetized, proestrous rats. Female rats were fitted with atrial catheters on diestrus. On proestrus, hourly blood samples were collected from 0900-2100h. At 1330h, rats received PB (40 mg/kg BW) to block hypothalamic LHRH release, or saline. Every 30 min from 1400-1800h, PB-treated rats received i.v. pulses of LHRH (15, 150, or 1500 ng/pulse) or saline, along with concurrent pulses of NPY (1 or 10 μg/pulse). Plasma samples were analyzed by LH RIA. In PB-treated rats receiving vehicle pulses only, LH surges were completely blocked. Pulsatile LHRH treatments at 15, 150 and 1500 ng/pulse produced sub-physiological, physiological, and supra-physiological LH surges, respectively. Simultaneous administration of NPY pulses with 15 ng/pulse LHRH produced significant, dose-related potentiations of LHRH-stimulated LH surges ($p<.0001$). NPY RIA of plasma confirmed NPY increments following treatments. These results support the hypothesis that NPY operates as a neuroendocrine modulator during generation of the preovulatory LH surge. (NIH HD20677, HD21921, HD00879, JEL; HD00727, MOD 1-1118, JKM)

393.4

INCREASED NPY CONTENT AND MONOAMINE TURNOVER IN THE ARCUATE NUCLEUS DURING LACTATION IN THE RAT. M.S. Smith, W.-S. Lee, C.R. Pohl, and G.E. Hoffman. Department of Physiology, University of Pittsburgh, Pittsburgh, PA 15261.

In response to the suckling stimulus, GnRH secretion is greatly inhibited, whereas prolactin secretion is increased. To investigate changes in hypothalamic function associated with lactation, we determined *in vitro* release rates and content of NPY and GnRH, as well as turnover rates of monoamines, in the median eminence (ME) and arcuate nucleus (ARC). Turnover rates were assessed by HPLC using α-MPT or pargyline. All studies were performed on day 10 lactation(8 pups suckling) or during diestrus of the estrous cycle. GnRH content and release in response to 55 mM K^+ did not differ between tissues from lactating and diestrous animals. However, NPY content was significantly increased in lactating rats when compared to diestrous animals: two-fold greater in the ME(7.5 ± 0.7 ng, site of terminals) and 8-fold greater in the ARC (320 ± 20 ng, site of cell bodies). The magnitude of release of NPY in response to 55 mM K^+ generally paralleled content. The differences in NPY activity were confirmed using immunocytochemistry. In the ARC from diestrous animals, only NPY-positive fibers were observed, whereas the ARC from lactating animals contained numerous NPY-positive cell bodies and fibers. The ARC from lactating rats also had increased turnover rates for NE, DA and 5HT. Interestingly, the increased 5HT activity could be blocked by inhibiting catecholaminergic activity.

The increased NPY content in the ARC during lactation could represent increased NPY neuronal activity or an inhibition of NPY release. The dramatic alterations in NPY and monoamine activity in the ARC may be involved in mediating suckling-induced changes in GnRH and prolactin secretion. (Supported by NIH grant HD 14643).

393.5

IMMUNODETECTION OF OPIOID INTERACTIONS AFFECTING NEUROENDOCRINE SYSTEMS IN MONKEYS. P.C. Goldsmith, K.K. Thind and J.E. Boggan*. Reproductive Endocrinology Center, Univ. California, Sch. of Med., San Francisco, CA 94143-0556.

We have previously shown that vasopressin (VP) and opioid (OP) neurons synapse directly on gonadotropin-releasing hormone (GnRH) neurons in juvenile monkeys. To investigate relationships between VP- and OP-immunoreactive (-IR) neurons, we performed double label immunostaining on vibratome sections through the SON and PVN of cynomolgus monkeys, whose neuroendocrine (NEU) neurons were pre-labeled (Soc. Neurosci. Abstr. 14:439, 1988). In the SON, beaded OP-IR fibers innervated about 33% of the VP-IR cell bodies anteriorly and 10% posteriorly. In the PVN, clusters of OP-IR terminals were grouped around major processes arising from about 42% of the VP-IR neurons. EM confirmed that OP-IR terminals made mostly symmetrical synapses on both NEU and non-NEU VP-IR cell bodies and dendrites.

These results provide anatomical evidence of direct OP modulation of NEU VP release from the median eminence and posterior pituitary, and of non-NEU, intrahypothalamic VP neuronal activity. We have now identified OP-IR innervation of VP, oxytocin, dopamine and GnRH neurons in the monkey hypothalamus. It is not known whether these OP-IR afferents arise from the same POMC neurons, whether they release the same POMC product at every synapse, or whether the peptides impart stimulatory or inhibitory post-synaptic effects. Other data have suggested CRF may coexist in some VP-IR neurons, and that CRF acts via VP neurons to stimulate OP neuronal activity and to inhibit GnRH release. Taken together, these results suggest that stressful stimuli initiate a complex combination of peptidergic neurointeractions which regulate endocrine responses to stress in primates.

393.6

ELEVATION OF PRODYNORPHIN mRNA LEVELS IN THE RAT OVARY BY GONADOTROPIN STIMULATION. A. H. Kaynard and M. H. Melner. Divisions of Neuroscience and Reproductive Biology, Oregon Regional Primate Research Center, Beaverton, OR 97006.

Opioid genes are expressed in the gonads and may perform important physiological functions in these tissues. These studies were conducted to elucidate whether gonadotropin induced changes in ovarian function can modulate the expression of prodynorphin (pD) mRNA. Pre-pubescent (26-day old) female rats were used to reduce the confounding influence of endogenous gonadotropin stimulation. Treatments consisted of sc injections of PMSG, an FSH agonist, and hCG, an LH agonist. Both hormones have long serum half-lives and the treatment groups were as follows: **CNT** - (n=10) vehicle on Day 0, collect ovaries on Day 2; **PMSG** - (n=8) 20 IU PMSG on Day 0, collect ovaries on Day 2; **hCG/1d** - (n=8) 20 IU PMSG on Day 0, 10 IU hCG on Day 2, collect ovaries on Day 3; **hCG/7d** - (n=8) 20 IU PMSG on Day 0, 10 IU hCG on Day 2, collect ovaries on Day 9; **hCG/14d** - (n=7) 20 IU PMSG on Day 0, 10 IU hCG on Day 2, collect ovaries on Day 16. These treatments produced ovarian conditions mimicking: no-change, follicular development, ovulation/luteinization, mid-pseudopregnancy and luteolysis/follicular development, respectively. Total RNA was isolated from the ovaries and size separated by electrophoresis through denaturing 1.5% agarose gels. Northern blot analysis, using a 1.7 kb cDNA fragment of the rat pD gene as a probe, revealed an ~2.2 kb form of pD mRNA in all ovaries examined. All hormonal treatments significantly increased the level of pD mRNA. The greatest elevation of pD mRNA was seen in the **hCG/1d** group (6.0-fold, $p<0.0005$), followed by **hCG/14d** (4.7-fold, $p<0.0005$), **hCG/7d** (2.4-fold, $p<0.0005$) and **PMSG** (1.4-fold, $p<0.025$). Thus hypertrophy and hyperplasia of ovarian tissues caused by gonadotropin stimulation enhances pD mRNA levels (ie: **PMSG**) and luteogenesis is associated with the greatest induction of PD mRNA levels (ie: **hCG/1d**). High levels of pD mRNA in the hCG/14d group may reflect the growth of a new cohort of follicles in the now sexually mature rats. These results support the hypothesis that pD may play a role in the growth, maturation and differentiation of ovarian tissues throughout the estrous cycle. Supported by NIH DK41035,RR00163, and ONR NN00014-90-J-1122.

393.7

AGING INFLUENCES THE DIURNAL PATTERN AND THE LEVEL OF PROOPIOMELANOCORTIN (POMC) GENE EXPRESSION IN THE ARCUATE NUCLEUS OF OVARIECTOMIZED (OVX) AND ESTRADIOL (E2)-TREATED RATS. K. Scarbrough, N.G. Weiland, J.M. Lloyd*, G.H. Larson*, S. Chiu* and P.M. Wise. Dept. Physiology, U. Maryland, Baltimore, MD 21201

Reproductive function, levels of LH and the ability of rats to respond positively to E2 decline with age. POMC gene expression may influence the level or pattern of LH secretion. Alterations in POMC gene expression may contribute to age-related changes in LH secretion. The purpose of this study was to determine whether the level of POMC mRNA or the diurnal pattern of gene expression changes with age in association with alterations in LH secretion. Young (3-4 mo), middle-age (9-11 mo) and old (17-19 mo) rats were OVX. One week later, half of each group received Silastic capsules containing E2 and rats were killed 2 days later at 0300, 1000, 1300, 1500, 1800 and 2300 h (n = 6-7 rats/time for each treatment). Brain sections were sliced and prepared for *in situ* hybridization (Wise et al, Mol Endocrinol, In Press). Slides were apposed to film and POMC mRNA levels were measured by quantitating the relative optical density within a fixed window placed over the arcuate nucleus. POMC mRNA levels were highest in young OVX rats. In this age group, E2 lowered the average level of POMC gene expression and induced a diurnal rhythm in which levels were highest at 0300h. POMC mRNA decreased in old compared to young rats in both E2-treated and OVX animals. Middle-aged rats exhibited an intermediate level of POMC mRNA. In addition, middle-aged and old E2-treated rats exhibited no detectable rhythm in POMC mRNA levels. In conclusion, our data demonstrate that aging influences both the average levels and the diurnal rhythmicity of POMC gene expression. These alterations may contribute to the age-related changes after LH secretion in OVX rats and in the ability of E2 to induce LH surges.

393.8

THE SEXUALLY DIMORPHIC DISTRIBUTION OF SUBSTANCE P IN SPECIFIC ANTERIOR PITUITARY CELL TYPES. Elaine R. Brown, Kevin A. Roth*, & James E. Krause. Depts. of Anatomy & Neurobiology and Pathology, Washington University School of Medicine, St. Louis, MO 63110.

Substance P (SP) immunoreactivity has been detected by radioimmunoassay in the rat anterior pituitary (AP). Its precise cytochemical location is of interest since SP may modulate pituitary hormone secretion locally. We have used a sensitive immunogold silver-enhancement staining technique to identify SP-immunoreactive (SP-ir) cells in the gland. Colocalization studies showed that the vast majority of SP-ir cells in the male AP were also immunoreactive for GH, representing approximately 25% of the somatotroph population in the male. SP-ir cells did not colocalize with lactotrophs, gonadotrophs, or corticotrophs; however, rare TSH/SP-ir cells were found in the male AP. Comparisons of pituitaries from males and females revealed that females have fewer SP-ir cells, and that only about 5% of the somatotrophs in the female express SP. This sexual dimorphism is diminished after 6 days of ovariectomy (OX), which results in a 3-fold increase in the GH/SP-ir cell population. This suggests that the previously reported estrogen-induced decrease in SP gene and peptide expression in the pituitary occurs in a subpopulation of somatotrophs. To test this hypothesis, the distribution of SP-ir cells was examined in pituitaries from oil- and estrogen-treated OX rats. Estrogen reduced the percentage of somatotrophs with SP immunoreactivity by 70% compared to OX oil-treated controls, indicating that estrogen regulates SP levels in the pituitary possibly by acting on a subpopulation of somatotrophs to suppress SP expression. Estrogen does not appear to alter the percentage of SP-ir cells colocalizing with TSH. These SP-expressing thyrotrophs were observed more frequently in the female than in the male, regardless of steroid status. These studies reveal that males have more total SP-ir cells in the AP than do females, and that there is a sexually dimorphic pattern of SP distribution in the gland. Males have a higher percentage of SP-ir GH cells, whereas females have more SP-ir thyrotrophs than do males. This localization of SP in the pituitary will aid in the investigation of the local regulation of pituitary hormone secretion. Supported in part by the Washington University-Monsanto Agreement.

393.9

GNRH NEURONS IN THE FETAL LAMB HYPOTHALAMUS ARE NOT SEXUALLY DIFFERENTIATED. RI Wood*, SW Newman, MN Lehman, DL Foster*, Reprod. Sci. Prog., Depts of Physiology, Anatomy & Cell Biology, Obstetrics & Gynecology, and Biology, The Univ. of Michigan, Ann Arbor, MI, 48109, and Dept of Anat. & Cell Biol., Univ. Cincinnati Coll. Med., Cincinnati, OH, 45267.

The critical period for sexual differentiation of the brain in the developing lamb occurs before birth. Exposure to steroids during mid-gestation alters the control of GnRH secretion after birth (Wood and Foster, unpublished). We have examined GnRH neurons in male and female lambs during the critical period to determine if sexual dimorphism of these neurons exists. The number and distribution of GnRH-containing neurons from mid-gestation (85 days) male and female fetuses were compared (n=3 each). Immunoreactive cells were labelled using LR-1 antiserum (R. Benoit) with the Vectastain ABC kit. GnRH neurons were localized in 60 um coronal sections in a block of tissue extending from the diagonal band of Broca to the rostral mammillary bodies. Neither the pattern of distribution nor the estimated number of GnRH neurons in male and female fetuses differed (1852±340 vs 1727±350, P>.05), and the total number of cells was similar to that reported for the adult ewe (Lehman, M. et al, *J Comp Neurol*, 244:19, 1986). Unlike the adult, substantial numbers of GnRH neurons in the fetus were present caudal to the optic chiasm (males 28% of total, females 37%, P>.05). These data indicate that the distribution of GnRH neurons may change during later stages of development, but is not sexually differentiated during the critical period. (Supported by USDA 89-37240-4561)

393.10

Properties of Neuroendocrine Cells Migrating from Olfactory Placode into Basal Forebrain. Zheng, L.-M., M. Schwanzel-Fukuda, J.F. Heitmancik, R.B. Gibbs, D.W. Pfaff. Laboratory of Neurobiology and Behavior, Rockefeller University, New York, N.Y. 10021.

LHRH-immunoreactive cells migrate from the developing olfactory placode into the basal forebrain (Schwanzel-Fukuda and Pfaff, Soc. Neuroscience, Abstr. p. 984, '88; Nature, 338:161-164, '89; Wray et al., Devel.Brain Res., 46:309-318, '89), associated with NCAM (Schwanzel Fukuda et al., Soc. Neuroscience Abstr. '90). The following experiments addressed the questions (A) do these immunoreactive neurons actually express the LHRH gene; and (B) is the migration specific to LHRH neurons among neuroendocrine cells?

A. In fetal mouse olfactory apparatus and forebrain, in situ hybridization was performed using ^{3}H-riboprobes for LHRH mRNA, and an established protocol (Gibbs et al., Mol. Brain Res., 6:275-287, '90). The antisense riboprobe revealed positive labelled cells just outside the olfactory placode on days 12 and 13, and these cells tended to be clustered. The control sense riboprobe did not yield labelled cells. We conclude that migrating LHRH-immunoreactive cells do express the LHRH gene.

B. Immunocytochemistry was performed in fetal mouse olfactory apparatus on days E11 through E13 using the following antisera: thyrotropin releasing hormone, corticotropin releasing hormone, growth hormone releasing hormone, oxytocin, vasopressin, neuropeptide Y and somatostatin. While the migration of LHRH neurons was confirmed, none of these other antisera revealed immunoreactive cells under the conditions tested. Among cells expressing neuroendocrine genes of interest, so far, LHRH neurons appear specifically to make this migration from olfactory placode to brain. Supported by NIH grant NS 19662 and funds from the Whitehall Foundation (M.S.F.).

393.11

INCREASED SUBSTANCE P AND NEUROKININ B GENE EXPRESSION IN HYPOTHALAMI OF POSTMENOPAUSAL WOMEN. N.E. Rance and W.S. Young III. Department of Pathology, University of Arizona College of Medicine, Tucson, AZ 85724, and Laboratory of Cell Biology, NIMH, Bethesda, MD 20892.

We have recently shown a striking hypertrophy of neurons containing estrogen receptor, Neurokinin B (NKB) and Substance P (SP) mRNAs in the infundibular nucleus of postmenopausal (POSTM) women (Rance et al., 1990, J Clin Endo Metab. In press; Rance and Young, 1990, Endo Soc Abst). In the present study, we determined if POSTM neuronal hypertrophy is accompanied by increased gene expression. Sections from hypothalami of premenopausal (PREM: N=3) and POSTM (N=3) women were incubated with [^{35}S]-labeled synthetic oligonucleotide probes complementary to NKB and SP mRNAs. The mean cross-sectional areas of POSTM infundibular neurons labeled with the SP and NKB probes increased to 180% and 196% of PREM values, respectively. Audioradiographic grain densities (#grains/100μm^2) for both SP (300%) and NKB (130%) were also elevated in the POSTM women. The most striking findings were 4-fold (SP) and 15-fold (NKB) increases in the numbers of labeled neurons/tissue area in the POSTM infundibular nucleus. These data support the hypothesis that POSTM neuronal hypertrophy is due to removal of the inhibitory feedback of ovarian steroids and demonstrate that human menopause is associated with marked increases in hypothalamic neuropeptide gene expression.

393.12

INTERLEUKIN-1 SUPPRESSES LH SECRETION WITHOUT CHANGING HYPOTHALAMIC GnRH RELEASE K.-Y. Francis Pau, Matthew J. Berria and Harold G. Spies, Division of Reproductive Biology, Oregon Primate Research Center, Beaverton, OR 97006.

Interleukin-1 (IL-1) has diverse roles in both peripheral tissue and brain. Intracerebroventricular infusion of IL-1 suppresses pituitary luteinizing hormone (LH) release. The presence of IL-1 and its receptors in the mediobasal hypothalamus (MBH) may indicate a hypothalamic mechanism for IL-1 influence in pituitary gonadotropin regulation. To address this issue we collected MBH push-pull (PP) perfusates for gonadotropin-releasing hormone(GnRH) and peripheral blood samples for LH estimates at 10- or 20-min intervals for 8h in ovariectomized (OVX) and ovarian intact (INT) rabbits. Recombinant human IL-1α (1 or 2 μg/h) or human IL-1ß fragment (IL-1ßf; aa163-171; 2 μg/h) was infused continuously into the MBH via the PP system for 4 h after an initial 4 h of PP perfusion. Plasma LH levels were reduced after MBH infusion of IL-1α at 1 μg/h (pre-ILα LH = 1.04 ± 0.25 vs post-ILα LH = 0.63 ± 0.18 ng/ml, $p<0.05$, n=6) and 2 μg/h (1.23 ± 0.31 vs 0.79 ± 0.22, $p<0.01$, n=6) in OVX does and at 2 μg/h (0.30 ± 0.06 vs 0.22 ± 0.05, $p<0.01$, n=7) in INT does. In contrast, MBH-GnRH in PP perfusates was not altered by IL-1α in these does even though GnRH was readily measurable (pre-ILα GnRH = 2.27 ± 0.14 vs post-ILα GnRH = 2.36 ± 0.26 pg/ml in OVXs, $p>0.05$, n=6). Infusion of IL-1ßf changed neither MBH-GnRH nor LH release in 3 OVX and 3 INT does. These results suggest that the ovarian-independent, inhibitory action of IL-1α on pituitary LH release is mediated by factor(s) other than release of MBH-GnRH. Supported by MRF8923, HD16631, HD07133 & RR00163.

BASAL GANGLIA AND THALAMUS VI

394.1

THE ROLE OF D1/D2 INTERACTION AND DOPAMINE RECEPTOR DENSITY IN BEHAVIORAL SUPERSENSITIVITY TO DOPAMINE AGONISTS. G.J. LaHoste, M. Andreini* and J.F. Marshall. *Dept. Psychobiology, Univ. California, Irvine, CA 92717.*

Evidence from several experiments necessitates a revision of the widely believed concept that dopaminergic supersensitivity can be explained primarily by increases in striatal D2 density. First, rats given a unilateral 6-OHDA lesion of the dopamine (DA) pathways and subsequent chronic treatment with the selective D2 antagonist eticlopride show vigorous rotation contralateral to the lesion despite the fact that D2 density is symmetrically up-regulated in the two striata. Second, chronic treatment of neurologically intact rats with haloperidol, which increases striatal D2 density to the same degree (25-40%) as a 6-OHDA lesion, results in a modest (2-fold) increase in sensitivity to the behavioral effects of the D2 agonist quinpirole or the mixed D1/D2 agonist apomorphine, in marked contrast to the 10- to 40-fold increase in sensitivity observed following 6-OHDA treatment.

Alternatively, the profound supersensitivity of 6-OHDA- or reserpine-treated rats to DA agonists may relate to the breakdown of the normal D1/D2 synergism. First, treatment with 5 daily injections of reserpine (1 mg/kg s.c.) induces a breakdown in the synergistic effects of D1 and D2 agonists but does not alter striatal D1 or D2 receptor density. Second, intact rats given a fixed, high dose (20 mg/kg i.p.) of the D1 agonist SKF 38393 show a dose-response relationship for stereotypy to quinpirole similar to that observed in reserpine-treated rats not given the D1 agonist.

Thus, two types of DA receptor supersensitivity can be discerned: a modest one associated with an increase in D2 receptor density, and a profound one that is not dependent on increased D2 or D1 receptor density and, instead, is associated with a breakdown in D1/D2 synergism. We hypothesize that the profound type of dopaminergic supersensitivity results from the liberation of this receptor subtype from control by the D1 receptor. This reformulation may have important implications for understanding certain aspects of tardive dyskinesia, Parkinson's disease, and schizophrenia.

394.2

PARALLEL INDUCTION OF JUN B AND C-FOS EVOKED IN THE STRIATUM BY THE PSYCHOMOTOR STIMULANT DRUGS COCAINE AND AMPHETAMINE. R. Moratalla,[1] H.A. Robertson,[2] P.A. DiZio,[3] and A.M. Graybiel.[1] [1]Dept of Brain and Cognitive Sciences, M.I.T., Cambridge, MA 02139, [2]Dept of Pharmacology, Dalhousie Univ., Halifax, Nova Scotia, [3]Spatial Orientation Lab, Brandeis Univ., Waltham, MA 02254.

We have shown by immunohistochemistry and in situ hybridization that the psychomotor stimulant drugs, amphetamine and cocaine, induce c-fos expression in the striatum (Graybiel et al., this meeting). This finding raises the question of what dimerization events occur in relation to the induction of c-fos. In several models of immediate early gene (IEG) induction (e.g., long-term potentiation, convulsion), sets of IEGs of the leucine zipper family are co-induced. To test whether members of the jun family are co-activated with c-fos in the striatum of psychostimulant-treated rats, we carried out in situ hybridization with oligonucleotide probes for jun B, c-jun and c-fos on tissue sections from rats treated intraperitoneally with cocaine (25 mg/kg) and amphetamine (5 mg/kg), and euthanized 1 hr later. We compared the patterns of mRNA induction in the striatum for the three IEGs in serial sections.

The in situ hybridization autoradiograms show strong induction of jun B by cocaine and amphetamine, and demonstrate that this induction was paralleled by activation of c-fos. By contrast, there was no evidence for induction of c-jun mRNA transcripts in the striatum of the same brains. These findings establish that, among IEGs of the leucine zipper family, at least two, jun B and c-fos, are coordinately induced in the striatum by psychomotor stimulants. These transcriptional events may be related to the extended effects of such drugs. Cortical lesions also coinduced jun B and c-fos in the ipsilateral cortex, suggesting a more general incidence of this pattern of IEG co-expression. Supported by The Seaver Institute, NIH 5RO1 25529-02, NARSAD, United Parkinson Foundation, and MRC of

394.3

SYNERGISTIC ACTIVATION OF THE IMMEDIATE-EARLY GENE c-FOS IN STRIOSOMES BY D1- AND D2-SELECTIVE DOPAMINE AGONISTS. M.L. Paul[1], A.M. Graybiel[2] and H.A Robertson[1]. [1]Dept. of Pharmacology, Faculty of Medicine, Dalhousie University, Halifax, N.S., Canada B3H 4H7 and [2] Dept. Brain & Cog. Sci., MIT, Cambridge, MA 02139, U.S.A.

D1- and D2-selective dopamine agonists interact synergistically to induce rotation in rats with unilateral 6-OHDA lesions (Robertson, G.S. and Robertson, H.A., Brain Res. 384:384, 1986). Systemic injections of D1-dopamine selective agonists (but not D2-selective agonists) activate the immediate-early gene c-fos in striatum ipsilateral to a 6-OHDA lesion (Robertson, H.A. et al, Brain Res. 503:346, 1989). Accordingly, we asked what would happen to c-fos activation if we administered a low dose of a D1-selective agonist together with a D2-selective agonist. Rats with a unilateral 6-OHDA lesion were given either SKF-38393 (0.5 mg/kg, i.p.), LY-171555 (0.25 mg/kg, i.p.) or the combination. Rotation was monitored for 2 hours, following which the rats were perfused for immunohistochemical localization of c-fos protein and calbindin 28kD.

The D1-agonist SKF-38393 produced little rotation and little c-fos activation. LY-171555 produced little or no c-fos activation as expected, even though this low dose caused turning. Although LY-171555 produced little or no c-fos activation by itself, the combination of the D1 and D2 agonists produced marked c-fos activation. Both the c-fos activation and rotation were reversed by MK-801 (1 mg/kg i.p.). In animals receiving both the D1 and D2 agonists together, much of the c-fos immunoreactivity was located in striosomes.

(Supported by MRC of Canada and Javits NIH NS 25529).

394.4

REGULATION BY D1 AND D2 DOPAMINE RECEPTORS OF STRIATONIGRAL AND STRIATOPALLIDAL PEPTIDE mRNA LEVELS. C.R. Gerfen, T.M. Engber, Z. Susel, L.C. Mahan, F.J. Monsma, Jr., L.D. McVittie and D.R. Sibley. Lab of Cell Biology, National Institute of Mental Health and Experimental Therapeutics Branch, NINDS, National Institutes of Health, Bethesda, MD 20892.

We examined the differential regulation through D1 and D2 dopamine receptor subtypes of peptide expression in striatopallidal and striatonigral neurons. *In situ* hybridization histochemistry shows that striatal neurons that project to the globus pallidus express both enkephalin and D2 dopamine receptor mRNA whereas striatal neurons that project to the substantia nigra express mRNA encoding the peptides dynorphin and substance P and also express the D1 dopamine receptor. Unilateral lesions of the nigrostriatal dopamine pathway with 6-hydroxydopamine (6-OHDA) in rats results, on the lesioned side, in a marked elevation in enkephalin mRNA, a decrease in substance P mRNA and no significant change in dynorphin mRNA in striatal neurons. Lesioned animals that are later treated for 21 days with the D1 specific agonist SKF-38393 show, on the lesioned side, an elevation of both dynorphin and substance P mRNA expression in striatonigral neurons but no change in the lesion-induced increase in enkephalin mRNA expression in striatopallidal neurons. Lesioned animals that are treated instead with the D2 specific agonist quinpirole show, on the lesioned side, a reduction in the lesion-induced increase in enkephalin mRNA expression in striatopallidal neurons but no change in either substance P or dynorphin mRNA expression in striatonigral neurons. These data show that the differential affect of dopamine on striatonigral and striatopallidal output pathways are mediated by the D1 and D2 dopamine receptor subtypes, respectively.

394.5

EXPRESSION OF GFAP AND J1/TENASCIN IN NORMAL DEVELOPING NEOSTRIATUM AND AFTER 6-OHDA LESION. T.F. O'Brien, K. Harrington*, A. Faissner, M. Schachner, and D.A. Steindler. Dept. of Anat. and Neurobiol., Univ. of Tenn., Memphis, and Dept. of Neurobiol., Univ. of Heidelberg.

The neostriatum is composed of two compartments contributing to structural and functional organization. Recently, lectinophilic molecules have been identified in the interface of the patch-matrix compartments during development (Steindler et al JCN 267:357, 1988). The present study was undertaken to assess the role of glia (by immunoreactivity for GFAP), associated glycoconjugates (by anti-J1/tenascin), and afferent input [by TH immunoreactivity and 6-OHDA lesions of the substantia nigra (SN)] in neostriatal development. Adjacent sections from ICR mice on postnatal day 1-12 were processed by routine histochemical and immunohistochemical procedures using AChE and TH as mosaic markers. J1/tenascin outlines the interface of the patch-matrix system. Both J1/tenascin and GFAP are more prominent in the matrix compartment which may reflect a later differentiation of this compartment compared to the patches. GFAP immunoreactive cells with numerous processes are present in the patch-matrix interface and it is from these cells that matrix molecules like J1/tenascin may emanate. Lastly, neonatal 6-OHDA lesions of the SN results in a marked depletion of J1/tenascin immunostaining of the neostriatum on the lesion side. Thus the expression of recognition molecules like J1/tenascin may be under the influence of afferent innervation. Supported by USPHS grant NS 20856, NSF grant BNS-8911514 and the DFG.

394.6

A COMPARISON BETWEEN DORSOLATERAL AND VENTROMEDIAL STRIATAL PATHWAYS THROUGH THE MONKEY BASAL GANGLIA. S.N. Haber, E.L. Lynd, S.J.Mitchell+ Department of Neurobiology and Anatomy, University of Rochester, Rochester, N. Y., +VA Medical Center, Syracuse, N.Y.

We compared the output pathways from the dorsolateral (motor) striatum and the ventromedial (limbic) striatum to the globus pallidus and substantia nigra and further traced the output pathways from the different pallidal regions that receive these two inputs. Injections of 3H amino acids, PHA-L, or cholera toxin, subunit B were placed in the dorsolateral putamen or ventral striatum. Processed sections were double stained for NGF receptors to determine the overlap of tracers with cholinergic neurons. Tracer injections were then made in regions of the globus pallidus that receive either motor or limbic input.

Results showed that terminals from functionally different regions of the striatum are strictly segregated in the pallidum. Ventral striatal projections overlap extensively with neurons of the N. Basalis, while those from the dorsal striatum do not. Projections to the nigra are also topographically organized, however, patches of terminals from each region of the striatum are observed within the domain of the terminals arising from the other injection site. Furthermore, while projections from the dorsal pallidum terminate primarily in the ventral portions of the substantia nigra and ventral pallidal projections terminate primarily in the dorsal substantia nigra, some overlap between dorsal and ventral pallidal terminals are observed in the substantia nigra. These results suggest that pathways from the motor and limbic-related striatum maintain segregated pathways in the globus pallidus, however some integration of these functionally different regions appears to take place in the substantia nigra.

394.7

A DYADIC PATCH-MATRIX SCHEME DOES NOT ADEQUATELY DEFINE THE HISTOCHEMICAL HETEROGENEITY OF THE HUMAN STRIATUM. D. G. Cole and N. W. Kowall. Neurology Service, Massachusetts General Hospital, Boston MA 02114.

The generally accepted contemporary view of striatal organization designates two compartments based on histochemical and hodological criteria: the patch, or striosome, and the surrounding matrix. This paradigm is mainly based on observations in the rat and cat. In our studies of human striatum we have been impressed by the complexity of substance P (SP) and met-enkephalin (ME) staining patterns, both considered to be classical markers for striatal patches. Serial sections of normal human striatum at the level of the nucleus accumbens were stained for acetylcholinesterase (AChE), NADPH diaphorase, tyrosine hydroxylase (TH), calbindin D28K, ME, SP, cholecystokinin (CCK), transforming growth factor alpha (TGF), and MAP-2. SP, ME, TGF, and CCK all showed a similar core of low immunoreactivity surrounded by a rim of intense staining. Dorsally these patches were smaller and were often cut tangentially resulting in a small immunoreactive rim without a core. A superimposed gradient of matrix staining was maximal ventrally where it merged with the immunoreactive rim of the patches from which it could not be distinguished. Calbindin staining is uniform in the matrix without a dorsoventral gradient. Patches of low calbindin staining correspond exactly with the unstained cores of SP, ME, TGF, and CCK. As previously reported, the areas of low AChE, NADPH diaphorase, MAP-2 and TH correspond. These patches, however, are larger than the calbindin patches and correspond exactly to the core plus immunoreactive rim of SP, ME, TGF, and CCK staining. The core region of patches did not stain positively with any method. Our results show that striatal organization in the human is more complex than previously reported. The neurochemical characteristics of the core region remains to be defined.

394.8

ALL SERIALLY ANALYZED CHOLINE ACETYLTRANSFERASE IMMUNOREACTIVE NEURONS SHOW DIRECT SOMATIC OR DENDRITIC CONTACTS WITH UNLABELED SPINY NEURONS IN ADULT RAT CAUDATE-PUTAMEN NUCLEUS. V.M. Pickel & J. Chan*., Div. Neurobiol., Dept. Neurol. & Neurosci., Cornell U. Med. Coll., NY, NY 10021.

Direct somatic and dendritic contacts between neurons mediate electrical coupling and influence the level of expression of choline acetyltransferase (ChAT) in certain neurons. To determine the relative frequencies of contacts between cholinergic and non-cholinergic neurons in the adult rat striatum, we examined the ultrastructural localization of ChAT-antibodies in serial ultrathin sections using the peroxidase-antiperoxidase (PAP) labeling method. Of 24 ChAT-labeled soma, 21% were in direct apposition to unlabeled soma. The remaining 79% of the ChAT-labeled perikarya appeared more bipolar and were not in apposition with unlabeled soma, but did show somatic junctions with unlabeled dendrites and dendritic appositions with unlabeled soma and dendrites. Appositional zones between plasmalemmas of ChAT-immunoreactive and unlabeled soma and dendrites were characterized by: 1) absence of glial processes, 2) parallel spacing with intermittent separation and fusion or occasionally parallel arrays of thin (1-2 nm) electron-dense bands, and 3) proximity of organelles such as subsurface cisternae and saccules of rough and smooth endoplasmic reticulum which may be involved in calcium-storage in both labeled and unlabeled cells. The observed contacts may serve as a substrate for either electrotonic coupling or more long-term changes involving second messengers. (Supported by grants from NIDA (DA04600) and NIH (HL18974)).

394.9

3D FUNCTIONAL NEUROANATOMY OF HAND MOVEMENTS IN HEMINEGLECT. R.K. Deuel[+], E.M. Santori[Φ], and A.W. Toga[Φ]. [+]Depart. of Ped., Wash. Univ. Sch. of Med., St. Louis, MO 63110 and [Φ]Depart. of Neurol., UCLA, Los Angeles, CA 90024.

In hemineglect after frontal cortical damage there is decreased neural activation in motor thalamus and basal ganglia as determined by quantitative 2-deoxyglucose autoradiography (2DG) in the monkey. The effect of continuous manual motor behavior on 2DG uptake by these structures was tested in neglect monkeys exerting cued, measured, and timed forces with the hands. 2DG uptake was brisk and symmetrical in supplementary, cingulate, and primary motor cortices with decrements in anterior thalamus that included regions of reticularis, nVA, and nVL, and in parts of basal ganglia. Computerized three dimensional reconstructions of paired histology and 2DG images were used to more precisely localize decrements. These were confined to narrow subregions of motor thalamic nuclei in pulling monkeys. In comparison thalami from non-pulling neglect animals showed 2DG decrements that conformed much more closely to conventional nuclear boundaries. Thus, manual motor activity induces neural activation in sectors of thalamus that are abnormally quiescent during resting neglect. The findings show that synaptic activity in hemineglect is regulated by motor behavior within the constraint imposed by anatomic connectivity.

394.10

THE NORADRENERGIC INNERVATION OF THE THALAMIC RETICULAR NUCLEUS AND THE BASAL NUCLEUS REGION IN RATS. C. Asanuma. Laboratory of Neurophysiology, NIMH, NIH Animal Center, Poolesville, MD 20837.

While the axonal arbors of thalamocortical and corticothalamic collaterals dominate the neuropil of the thalamic reticular nucleus (TRN), recent attention has focused upon some of the other inputs to the TRN. This is because these additional inputs may indirectly influence the state dependent gating of signal flow through the nuclei of the dorsal thalamus. Neurons in several sites afferent to the TRN, including the locus coeruleus and the basal nucleus, have discharge properties which are closely correlated with changes in arousal levels. In the present study, dopamine-β-hydroxylase (DBH) immunohistochemistry was used to examine the arborization patterns of noradrenergic axons within the TRN and the basal nucleus region. This was combined with intracellular injections of Lucifer yellow (LY) in the lightly fixed slice preparation in some cases to examine the structural relation of noradrenergic axons to neurons within these two areas.

Within the TRN, many DBH+ axons are present. These generally extend linearly, giving rise to boutons *en passant* which are rather evenly dispersed throughout the nucleus. When TRN neurons are injected with LY, DBH+ axons are seen in relation to dendrites of all diameters, with the *en passant* DBH+ boutons forming random apparent appositions with dendrites they intersect. Within the basal nucleus region, however, the DBH+ axons, while also giving rise to some *en passant* boutons, frequently emit focal bouton clusters. When cells in the basal nucleus region are injected with LY, such DBH+ bouton clusters are seen in close juxtaposition with the somata and proximal dendrites of some, though not all, of the neurons within the region. The noradrenergic axonal boutons, therefore, are randomly dispersed within the TRN, but are selectively aggregated upon some neurons within the basal nucleus region.

394.11

INTRACELLULAR AUTOSTIMULATION OF *IN VITRO* GUINEA-PIG THALAMIC NEURONS (TH) UTILIZING A HARDWARE BIO-ELECTRIC RE-ENTRY SYSTEM. Y. Yarom[1] and R. Llinás, Dept. of Physiol & Biophysics N.Y.U. Med. Ctr, NY, N.Y., Dept. of Neurobiology, Hebrew University, Jerusalem Israel[1]

A waveform generator simulating the feedback activity from the reticular thalamus was constructed to explore the role of hyperpolarizing potentials in the generation of oscillatory thalamic activity. IPSP-like waveforms were simulated by controlling the amplitude and rise and fall time for current injected through the recording electrode. The injection, was triggered by TH spikes and was proportional to the number of spikes in the burst. The "IPSP" parameters best suited to supporting rhythmic activity were then determine. **Low frequency** (6 to 10Hz) spindle-like oscillations were elicited from cells at rest or slightly hyperpolarized (-65mV), if the duration of the hyperpolarizing current was long enough (> 50ms) to activate the calcium-dependent low threshold condutance (LTC). Given the above, a brief depolarization of TH neurons could trigger prolonged rhythmic activity at a frequency determined by the duration of the hyperpolarizing pulses. This activity usually lasted for 3-5 cycles, but could persist for 50 cycles. The interburst interval limited the burst duration. Thus, as the interval progressively shortened, the LTS decreased ending the burst—independently of its initial frequency. In contrast, if TH cells were held depolarized, inhibitory feedback could induce high frequency oscillations, by modulating the repetitive firing into well ordered trains whose frequency (10 to 50Hz) depended on the amplitude and duration of the "IPSPs". Thus: 1) Feed-back hyperpolarizing currents are necessary and sufficient to generate and maintain thalamic neuron rhythmicity *in vitro*, the oscillatory frequency being related to the resting potential level and IPSP duration. 2) The intrinsic membrane properties of thalamic neurons are sufficient to account for the transient nature of thalamic oscillations. Supported by 13742 from NINDS.

394.12

INTRACELLULAR RESPONSE OF RAT NUCLEUS ACCUMBENS NEURONS FOLLOWING STIMULATION OF HIPPOCAMPAL INPUTS IN AN IN VITRO SLICE PREPARATION. C.M.A. Pennartz and S.T. Kitai, Department of Anatomy and Neurobiology, College of Medicine, The University of Tennessee, Memphis, TN 38163.

The aim of the present study was to analyze responses of neurons of the nucleus accumbens to stimulation of the fornix, which contains afferents from the hippocampal formation. Using electrodes filled with 0.5 M K-methylsulphate and 2 % biocytin, recordings were made from 52 neurons (membrane potential: -68 ± 1 (SEM) mV, input resistance 42 ± 4 MOhm). After histological processing with avidin-Texas Red, 45 labeled neurons were identified as medium spiny neurons. A depolarizing postsynaptic potential (DPSP) following stimulation of the fornix was recorded in 48 neurons. Using intracellular injection of positive DC current and Na-channel blocker QX-314, this DPSP could be dissociated into an EPSP reversing its polarity at -11 to +15 mV and a bicucullin-sensitive IPSP reversing at -70 to -50 mV. The threshold for the IPSP was lower than for the EPSP. Furthermore, the IPSP onset latency was consistently shorter than the spike latency.

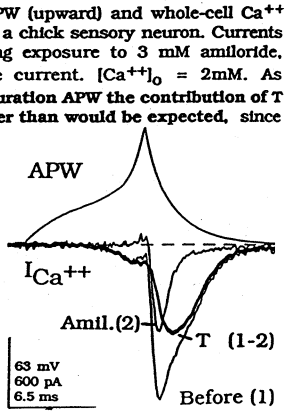
The data indicate that medium spiny neurons receive excitatory monosynaptic inputs from the subiculum. In addition, fornix stimulation most likely activates a feed-forward inhibitory pathway in the nucleus accumbens. (Supported by Grant USPHS NS 233886 and 20702 to S.T. Kitai).

CALCIUM CHANNELS III

395.1

ACTIVATION OF CA^{++} CURRENTS BY VOLTAGE-CLAMP COMMAND PULSES IN THE SHAPE OF ACTION POTENTIALS. **D.P. McCobb and K.G. Beam. Dept. of Physiology, Colorado State University, Ft. Collins, CO 80523.**

We have used action potential waveforms (APW's) as voltage-clamp command pulses to investigate Ca^{++} current activation by voltage transients which are more natural than conventional square steps. Traces below represent APW (upward) and whole-cell Ca^{++} currents (downward), recorded in a chick sensory neuron. Currents were recorded before and during exposure to 3 mM amiloride, which selectively blocks T-type current. $[Ca^{++}]_o$ = 2mM. As illustrated, with a normal, brief duration APW the contribution of T current to the total is much larger than would be expected, since when currents are activated with conventional voltage steps T is much smaller than the high-voltage activated current (HVA.≈N+L). Two factors can account for this: lower threshold and slower deactivation kinetics of T. The latter leaves T channels open after repolarization, when the driving force is great. We also find that increasing APW duration causes a much larger increase in HVA than in T current. Supported by NS08373 and NS26416.



395.2

Ca CHANNELS AND DIHYDROPYRIDINE-SENSITIVITY IN RAT CEREBELLAR GRANULE CELLS IN CULTURE. A. Feltz*, M. De Waard*, J.L. Bossu*, L. Fagni*, F. Tanzi*, P. Feltz. Laboratoire d'Etude des Régulations Physiologiques, CNRS, 23 rue Becquerel, 67087 Strasbourg, France.

A voltage-dependent Ca current can be elicited in cerebellar granule cells mechanically dissociated at PN5 and maintained 5 days in culture (DMEM + 25 mM KCl). With 10 mM $BaCl_2$ in the external medium, a current was elicited above -20 mV and reached a mean peak value of 150 pA at +10 mV. The total current (up to 90%) was half inactivated by holding the membrane potential at -70 mV. It was further stable for a 15 min period which allowed its pharmacological characterization, provided 1 mM ATP was added to the recording solution (pCa 8, 30 mM EGTA/CsOH, pH 7.2). Since full inhibition by nicardipine was attained at a concentration of 10 μM (IC50: 1 μM), we looked for a channel activity sensitive to the dihydropyridines (DHP) as recorded in cell attached condition (110 mM $BaCl_2$). Two channel types displayed such a sensitivity. A ≈ 25 pS L-type channel was recorded: its low opening probability in control conditions (<0.001) was greatly enhanced by BAY K 8644. In addition, a spontaneous 5-7 pS was observed at potentials above -50 mV which open time was greatly enhanced by both depolarization and addition of 10^{-6} M (-)BAY K 8644 (up to 300 ms at 0 mV). The latter channel might account for the DHP-sensitive Ca entry commonly measured in flux experiments.

395.3

VOLTAGE-DEPENDENT CALCIUM CURRENTS IN RAT CEREBELLAR GRANULE NEURONS (CGN). M. Bertolino, S. Vicini, R. Llinas* and E. Costa. FGIN, Georgetown University, Washington, D.C. 20007; *Dept. Physiol. and Biophysics, New York Univ. Med. Center, New York, NY 10016.

In order to learn about the biophysical and pharmacological properties of Ca^{2+} channels present in CGN, we monitored transmembrane currents in whole-cell configuration using the patch clamp technique. Currents were elicited from a holding potential of -90 mV in the presence of $BaCl_2$ 20 mM. Cadmium (100 μM) blocked completely the Ca^{2+} current, while neither amiloride (500 μM) nor w-conotoxin (10 μM) affected the current. While nifedipine (300 nM) partially blocked the current, the toxin from the funnel-web spider venom (FTX) (Llinas et al., PNAS, 1989, 89:1689) blocked most of the current.

Single-channel experiments, performed in cell-attached configuration with 110 mM $BaCl_2$, revealed the presence of a main conductance level of about 20 pS (whose frequency was increased when BAY K 8644 1 μM was added to the bath solution) and in some cases other lower conductance levels. Biophysical and pharmacological properties suggest that in cultured CGN at least two Ca^{2+} channel species can be detected. One can be characterized biophysically as L-type (Nowicky et al., Nature, 1985, 316:440) and the other with a lower conductance is similar to the P channel described by Llinas et al. (PNAS, 1989, 89:1689).

395.4

VOLTAGE-DEPENDENT AND PHARMACOLOGICAL PROPERTIES OF CALCIUM CURRENTS IN ISOLATED RAT CEREBELLAR PURKINJE CELLS.

P.E. Hockberger and L. Yousif. Dept. of Physiology, Northwestern Univ. Medical School, Chicago, IL 60611.

Whole-cell calcium currents were recorded from identified Purkinje cells acutely isolated from 1-2 week-old rats, as described previously (Hockberger et al., Soc. Neurosci. Abstr. 15: 1149, 1989). Both high-threshold (HT) and low-threshold currents were found although the latter did not appear until the second postnatal week. This report focuses on the properties of the HT current. The current amplitude was dependent upon the holding potential, e.g., peak amplitude was 50-75% larger when held at -80mV compared with -40mV. Regardless of the holding potential, the threshold for activation was -20mV (± 5mV) and the peak amplitude was evoked around 0mV (± 5mV). During voltage steps the degree of current inactivation was <10% during the initial 50ms, but it reached 20-40% after 400ms. Recovery from inactivation was slow (T = 1-2 min). Local application of calcium channel antagonists via micropipet revealed that nifedipine (NIF), w-conotoxin-GVIA (CTX), or a synthetic analog of funnel-web spider toxin (FTX)# reduced the HT current in a dose-dependent and reversible manner. Reductions of 80-100% were obtained when applying NIF, CTX, or FTX at 10^{-6}M and stepping from either -40 or -80mV. Including 8-bromo-cGMP (10^{-3}M) in the internal solution did not affect any of the measured parameters listed above.

This research was supported by NIH grants NS-26915 and NS-17489. #The FTX was kindly provided by Pfizer Central Research (Groton, CT) and Natural Product Sciences (Salt Lake City, Utah).

395.5

ISOLATION OF A CALCIUM CHANNEL FROM MAMMALIAN CEREBELLAR TISSUE USING SYNTHETIC FTX. B. D. Cherksey, M. Sugimori and R. Llinás Dept. of Physiology and Biophysics, NYU. Med. Ctr., New York, NY 10016.

FTX, the specific blocker of the P-type calcium channel was isolated from the venom of agelenopsis aperta is polyamine-like (Cherksey, et al ,Neurosci. Soc. Abs, 1989) and has been used to isolate a neuronal membrane protein which when reconstituted into a lipid bilayer gave channel activity. Small amounts of purified FTX made further biochemical studies unfeasible. Based on our structural analysis of FTX, we have synthesized a polyamine ("synthetic FTX") (Cherksey, et al, Biol. Bull, 1989) with specific P-channel blocking characteristics. The availability of synthetic FTX has made possible further studies of the P-channel protein. Synthetic FTX was coupled to Sepharose. Membranes from cow cerebellum homogenate, solubilized in 3% sodium choleate, were subjected, batch-wise, to affinity chromatography on Synthetic FTX gel. Bound protein was eluted with 1 M calcium chloride. The volume was reduced. Portions of the material were taken and reconstituted into lipid vesicles. Functional activity was determined using the lipid bilayer technique. The purified protein had channel-like activity, blocked by FTX, indistinguishable from that obtained using purified FTX as the gel ligand. SDS polyacrylamide gel electrophoresis under non-reducing conditions, gave a single sharp band in the 120,000 Da. region. Similar results were obtained using size-exclusion HPLC. Under reducing conditions only 2 bands were seen using SDS-PAGE: one sharp band at 70,000 Da. and a broad band over the 22,000 - 30,000 region. On HPLC, the broad band was resolved into 2 bands: 27,000 and 24,000 which could represent either two distinct proteins or one protein with differing states of glycosylation. These results differ substantially from those reported for the other known calcium channels. The purified protein was injected into rabbits and immune serum obtained. The antibody was found to react specifically with the P-channel protein. Both the functional activity and structural characteristics of the isolated P-channel protein differ from those reported for the L and N type channels and strengthen the identification of the P-type channel as a distinct molecular entity.

395.6

NEW ω-AGATOXIN CALCIUM CHANNEL ANTAGONISTS FROM FUNNEL WEB SPIDER VENOM ACTIVE IN THE AVIAN AND MAMMALIAN BRAIN.

V. J. Venema* and M. E. Adams. Dept. of Entomology, Univ. of California, Riverside, CA 92521.

Venom of the funnel web spider *Agelenopsis aperta* is a rich source of voltage-sensitive calcium channel (VSCC) antagonists known as ω-agatoxins. First recognized for their inhibition of presynaptic VSCC at insect neuromuscular junctions, the ω-agatoxins also are potent and selective antagonists of VSCC in vertebrate excitable tissue (see also Leibowitz *et al.*; Mintz *et al.*, this meeting). Reversed-phase liquid chromatography (RPLC) of crude *A. aperta* venom yields a broad ω-agatoxin elution profile characterized by inhibition of specific ω-conotoxin GVIA (ω-CgTx) binding to chick synaptosomal membranes. Using this inhibition as an assay for purification, we isolated ω-Aga-IIIA, an 8.5 kDa polypeptide constituting about 1% of total protein in the venom. ω-Aga-IIIA causes dose-dependent inhibition of ω-CgTx binding (EC_{50} = 10-20 nM) to a maximum of 100% above 60 nM. ω-Aga-IIIA also inhibits potassium-induced synaptosomal ^{45}Ca flux with an EC_{50} of 1-5 nM. However, saturating concentrations of ω-Aga-IIIA block only 67% of total flux, while ω-CgTx blocks 100% of the flux response. Therefore, 25-30% of total ^{45}Ca flux in chick synaptosomes appears to be insensitive to ω-Aga-IIIA.

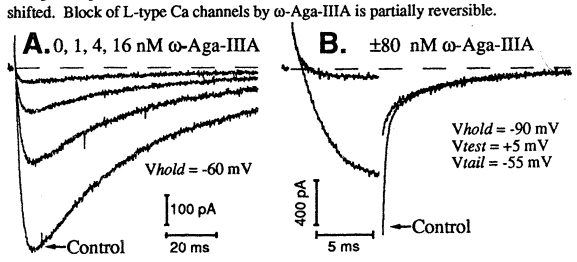
Potassium-induced ^{45}Ca flux in rat brain synaptosomes is relatively insensitive to ω-Aga-IIIA and ω-CgTx antagonism. However, new ω-agatoxins currently under study suppress rat synaptosomal ^{45}Ca flux at apparent nanomolar concentrations. Further analysis of the specificities and modes of action of the ω-agatoxins promises to yield useful information on the subclassification as well as tissue and phylogenetic diversity of VSCC.

Supported by NIH grant NS24472.

395.7

SELECTIVE BLOCK OF ATRIAL L-TYPE Ca CHANNELS BY THE SPIDER TOXIN ω-AGA-IIIA. M.D. Leibowitz, T. Bale*, M.E. Adams§, V.J. Venema*§ and C.J. Cohen. Merck Sharp & Dohme Res. Labs, Rahway, NJ 07065 and §Dept. of Entomology, Univ. California, Riverside, CA 92521.

ω-Aga-IIIA is a polypeptide toxin from venom of the funnel web spider *Agelenopsis aperta* (Venema and Adams, Mintz et al.; this volume). This toxin blocks selectively L-type Ca channel currents in acutely isolated guinea pig atrial myocytes. Whole-cell L-type Ca channel currents (carried by 5 mM Ca or Ba) are blocked by ω-Aga-IIIA (IC50 ≤ 1 nM, Fig A). Toxin block is modified by the dihydropyridine Ca agonist (+)202-791. In the absence of (+)202-791, 80 nM ω-Aga-IIIA blocks only ≈80% of the L-type Ca current and no slowly deactivating (T-type) Ca current (Fig B). However, in 100 nM (+)202-791, 40 nM ω-Aga-IIIA blocks >98% of the L-type Ca current. Unlike therapeutically used Ca antagonists, block by ω-Aga-IIIA is voltage-independent; channel kinetics are not changed and channel availability is not shifted. Block of L-type Ca channels by ω-Aga-IIIA is partially reversible.

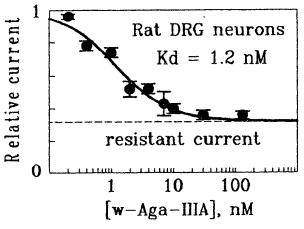


395.8

THE FUNNEL-WEB SPIDER TOXIN ω-AGA-IIIA BLOCKS N- AND L- TYPE CALCIUM CHANNELS IN NEURONS AND CARDIAC MUSCLE.

I. M. Mintz, V. J. Venema*, M. E. Adams, and B.P. Bean. Dept. of Neurobiology, Harvard Med. Sch., Boston MA 02115 and Dept. of Entomology, Univ. of California, Riverside CA 92521.

The peptide ω-Aga-IIIA from the venom of *Agelenopsis aperta* was tested on various calcium conductances in freshly dissociated neonatal rat DRG neurons, using whole cell recordings of Ba (5 mM) currents. The toxin blocked most high-threshold current with high potency (Kd= 1.2 nM) but left a fraction unaffected (figure). Occlusion experiments with ω-conotoxin GVIA (ω-CgTX, 3 μM) indicated that ω-Aga-IIIA (130 nM) suppressed both ω-CgTX-sensitive N-type current (n=10) and an additional ω-CgTX-insensitive fraction (n=9). Its efficacy on L-type current was studied using slowly-deactivating L current tails induced by BAY K 8644 (1 μM) in ω-CgTX (3 μM) pretreated cells. Such tails were abolished by 10 nM ω-Aga-IIIA (n=5).



ω-Aga-IIIA acted with similar potency on the N-type current of frog sympathetic neurons (n=20), the L-type current of rat ventricular cardiac cells (n=4), and high-threshold Ba currents in neonatal rat hippocampal neurons (n=3). In contrast, T-type currents in rat DRG neurons were not affected (200 nM ω-Aga-IIIA, n=3).

395.9

FUNNEL-WEB SPIDER TOXIN (FTX) SELECTIVELY BLOCKS THE SUSTAINED, BUT NOT THE TRANSIENT, CALCIUM CURRENT IN RETINAL HORIZONTAL CELLS J.M. Sullivan* and E.M. Lasater Depts. of Physiology and Ophthalmology, Univ. of Utah School of Medicine, Salt Lake City, Utah, 84108

Using the whole-cell patch-clamp technique, we have identified two separate Ca currents--one sustained ($I_{Ca}S$), the other transient ($I_{Ca}TR$)--in cultured horizontal cells (HCs) isolated from adult white bass retinas. Ca currents were enhanced using 10 mM extracellular Ca, while Na^+ and K^+ currents were pharmacologically supressed. The large transient Ca current is similar, but not identical, to the T-Current described in other preparations: $I_{Ca}TR$ activates above -60 mV, is inactivated at a holding potential of -40 mV and is carried less well by Ba^{++} than Ca. Unlike the T-Current, $I_{Ca}TR$ is not preferentially blocked by Ni^{++}. This is the first time that a large transient Ca current has been seen in HCs, or in a non-mammalian vertebrate preparation. The sustained Ca current is similar, but not identical, to the L-Current described in many preparations: $I_{Ca}S$ activates above -30 mV, is larger when Ba^{++} replaces Ca and is enhanced by the dihydropyridine agonist BAY K 8644 (Calbiochem). Unlike the L-Current, $I_{Ca}S$ is not preferentially blocked by Cd^{++}, nor is it reduced by w-conotoxin fraction GVIA (a gift from Dr. B. M. Olivera). FTX (a gift from Dr. R. Llinas), a factor isolated from the venom of the funnel-web spider (Llinas et al., *P.N.A.S.*, 86:1689, 1989), selectively blocks $I_{Ca}S$ at very low concentrations: a 1:300,00 dilution of the column fraction containing FTX blocked 60% of the sustained current, while a 1:10,000,000 dilution blocked 10% of this current. This block was maintained even after washing with FTX-free Ringer for up to 20 min. These results strongly suggest that the sustained and transient Ca currents are carried through two different and unique types of channels, and support the notion that there is a wide variety of Ca channels among different species and tissue types.

This work was supported by N.I.H. Grant EY05972 to E.M.L.

395.10

DISTINCT RAT BRAIN CLASS-C CALCIUM CHANNELS ARE GENERATED BY ALTERNATIVE SPLICING. W.J. Tomlinson*, M.M. Gilbert* and T.P. Snutch. Biotechnology Laboratory, Univ. of British Columbia, Vancouver, B.C., Canada V6T 1W5.

Rat brain class C Ca channel cDNAs (rbC) hybridize on Northern blots to two mRNAs of approx. 8 and 12 kilobases (kb). The deduced primary structure of two overlapping clones (rbC-61 and rbC-30) shows that the class C gene product is >90% identical to the rabbit cardiac dihydropyridine receptor/Ca channel (designated rbC-I). Analysis of a number of other cDNAs reveals distinct class C coding sequences (designated rbC-II). Further, in 1400 amino acids compared, rbC-I and rbC-II transcripts differ at only two sites. In the first instance, rbC-II transcripts have a three amino acid insertion (ProAlaArg) corresponding to the putative cytoplasmic loop between homology domains II and III. The second site, a 28 amino acid region which includes the third putative transmembrane segment (S3) of domain IV of rbC-I, is replaced by a different 28 amino acid hydrophobic stretch. By Southern blot analysis, rbC-I and rbC-II specific probes are shown to hybridize an identical pattern of rat genomic DNA fragments. In addition, polymerase chain reaction analysis of rat genomic DNA shows that the rbC-I and rbC-II transcripts are generated from the same genomic DNA fragment. These results suggest that in addition to the distinct genes which encode the rat brain class A, B, C and D Ca channels, alternative splicing provides a further source of Ca channel diversity in the CNS.

395.11

Biochemical Characterization of a High Affinity [^{3}H]Ryanodine Receptor From Rabbit Brain Membranes. P.S. McPherson and K.P. Campbell. Howard Hughes Medical Institute and Program in Neuroscience and Dept. of Physiology and Biophysics, University of Iowa College of Medicine, Iowa City, IA 52242.

The skeletal muscle receptor for the plant alkaloid ryanodine has been shown to be identical to the Ca^{2+} release channel of the sarcoplasmic reticulum. High affinity [^{3}H]ryanodine binding has been recently demonstrated in isolated brain membranes (Ashley, R.H., J. Memb. Biol., 111:179, 1989). We have shown that [^{3}H]ryanodine binding is enriched in membranes from the hippocampus but is significantly lower in membranes from the brain stem and spinal cord. Approximately 50% of [^{3}H]ryanodine labeled receptor is solubilized from brain membranes using CHAPS phosphatidylcholine containing 1 M NaCl. The brain [^{3}H]ryanodine receptor comigrates through sucrose gradients with the skeletal receptor as a large complex (~30S). Solubilized receptor is specifically immunoprecipitated by sheep polyclonal antibodies against purified skeletal muscle ryanodine receptor coupled to protein A Sepharose. [^{3}H]Ryanodine labelled receptor binds to heparin-agarose with high affinity, but does not bind to wheat germ agglutinin lectin. [^{3}H]Ryanodine labeled receptor is isolated using heparin-agarose chromatography followed by sucrose density gradient centrifugation. A protein of ~400,000 Da is enriched in peak [^{3}H]ryanodine binding-fractions of heparin-agarose eluates and sucrose gradients, and is cross-reactive with antibodies raised against the skeletal muscle ryanodine receptor. We propose that the ~400,000 Da protein is the brain form of the high affinity ryanodine receptor and that it functions as a Ca^{2+} release channel in brain endoplasmic reticulum. Kevin P. Campbell is an Investigator of the Howard Hughes Medical Institute.

395.12

DIVERSE ω-CONOTOXIN GVIA BINDING PROTEINS IN ELECTRIC ORGAN AND BOVINE BRAIN W.A. Horne, R.R. Delay, and R.W. Tsien Dept. of Molec. & Cell. Physiol., Stanford University School of Medicine, Stanford, Calif. 94305.

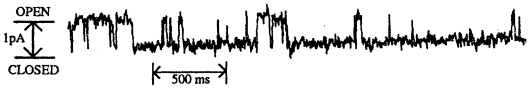
Binding sites for the marine snail toxin, ω-conotoxin GVIA (ωCgTx), have been identified in a variety of neuronal tissue types, and have been assigned exclusively to voltage-dependent calcium channels. ωCgTx has been shown to bind with high affinity (K_D=3 pM) to relatively few receptor sites (0.4 pmoles/mg protein) in bovine brain (Yamaguchi et al., *JBC* 263, 9491), and with low affinity (K_D= 1 μM) to a greater number of receptor sites (290 pmoles/mg protein) in synaptosomes prepared from the electric organ of *Discopyge ommata* (Miljanich et al., *Brain Res* 453, 247). Our results suggest that these binding sites represent a heterogeneous population of ωCgTx binding proteins and that, in electric organ, not all are actually calcium channels. Crosslinking experiments with the homobifunctional imidoester, dimethylsuberimidiate (DMS), reveal that ^{125}I-ωCgTx specifically labels two proteins in bovine brain with M_r values of 330 and 220 kD as determined by SDS-PAGE, and three proteins in electric organ synaptosomes with M_r values of 170, 60, and 45 kD. We have demonstrated that the 45 kD protein of electric organ is the α-subunit of the nicotinic acetylcholine receptor (nAChR) and that it accounts for a significant fraction of the total number of ωCgTx binding sites. Furthermore, it can be separated from both the 170 and 60 kDa proteins by immunoprecipitation with a Mab against the nAChR. All three electric organ ωCgTx binding proteins share similar pharmacological properties, in that half maximal displacement of ^{125}I-ωCgTx occurs in the range of 0.1-1.0 μM ωCgTx, and the binding of ^{125}I-ωCgTx is not inhibited by other calcium entry blockers, such as nitrendipine, or by α-bungarotoxin and other agents known to interact with the nAChR. ωCgTx binding both in brain and electric organ is characteristically inhibited by higher concentrations of Ca^{2+} (0.5-1.0 mM) whereas in brain preparations we demonstrate that ωCgTx binding is actually stimulated up to 3-fold at lower Ca^{2+} concentrations (0.1-1.0 μM). It is noteworthy that this stimulatory effect occurs over the same concentration range as Ca^{2+} binding to sites controlling permeation and inactivation of calcium channels.

395.13

DESIGN, SYNTHESIS, AND SINGLE CHANNEL CHARACTERIZATION OF A DIHYDROPYRIDINE-SENSITIVE CALCIUM CHANNEL PROTEIN.
A. Grove,†* J. M. Tomich†* and M. Montal.†
†UCSD, La Jolla, CA 92093 and *Children's Hospital, Los Angeles, CA 90027.

The major protein component of a dihydropyridine-sensitive calcium channel from skeletal muscle was cloned and sequenced (Tanabe, T. et al. 1987, *Nature* 328, 313-318). The primary structure suggests the occurrence of four internal repeats, each containing six presumably α–helical transmembrane segments. We designed a protein that mimics a pore-forming structure of the calcium channel; a carrier template (KKKPGKEKG) (Mutter, M. et al. 1988, *Tetrahedron*:44, 771-785) was used to direct the assembly of a bundle comprised of four identical 22-mer peptides with sequences corresponding to transmembrane segment IVS3 (DPWNVFDFLIVIGSIIDVILSE). This synthetic protein forms cation-selective channels in lipid bilayers. At pH 7.2, the single channel conductances in symmetric 50 mM $CaCl_2$, $BaCl_2$ and $SrCl_2$ are 7.2 ± 1.3, 9.5 ± 1.2 and 6.4 ± 0.4 pS, respectively, and 10.8 ± 1.9 pS in 500 mM NaCl. Channels are blocked by 10^{-7} M nifedipine, 10^{-6} M verapamil and 10^{-4} M of the local anesthetic analogue of lidocaine QX-222. A different synthetic protein containing four peptides with sequences corresponding to segment IVS5 (YVALLIVMLFFIYAVIGMQMFGK) does not form discrete channels.

Supported by NIGMS (GM-42340), NIMH (MH-44638 and MH-00778) and ONR (N00014-89J-1469).



Single channel current recorded at 100 mV in symmetric 50 mM $BaCl_2$, pH 7.2.

396.1

CONANTOKINS: STRUCTURE, FUNCTION AND DEVELOPMENTAL SPECIFICITY. L. J. Cruz, F. C. Abogadie*, B. M. Olivera, J. M. McIntosh, J. F. Hernandez* and J. Rivier*. Marine Science Inst., Univ. Philippines, Diliman, Q. C. 1101; Dept. of Biology, Univ. Utah, Salt Lake City, UT 84112; Salk Institute, La Jolla, CA 92057.

Fish-hunting marine snails produce numerous biologically active peptides; of particular interest are conantokins which inhibit the glutamate receptors of the NMDA subtype. First detected as components which induce sleep in young mice but cause hyperactivity in older mice, the peptides are structurally unique in having at least 4 residues of γ-carboxyglutamate (Gla) out of 17 to 27 amino acids. At present, 12 natural and synthetic conantokin analogs have been synthesized. A new peptide (conantokin R) with a definite structural homology to conantokins G and T has been characterized from *Conus radiatus* venom. Although only 7/27 amino acids are identical to the other conantokins, it has the conserved sequence Gly-Glu-Gla-Gla at the amino terminus. Comparison of the biological activities of several conantokins suggests that different NMDA receptor targets may be responsible for the sleep and hyperactivity symptoms. Synthetic conantokin analogs indicate that Gly at position 1 and Glu at position 2 are necessary for full potency; subtitution of Glu^2 by a Gln or an L-Asp inactivated the peptide and the D-Asp^2 analog produced an effect only in old mice at high dose. [Ser^1]conantokin G apparently has a normal sleeper activity in young mice but it is relatively inactive in older mice. Thus the conantokins may be useful probes for changes in NMDA receptor subtypes as a function of development. (Supported by GM 22737 and funds from the Marine Science Institute, U. P.)

396.2

CONANTOKINS: PEPTIDE PROBES OF THE NMDA RECEPTOR. R. A. Myers*, J. Rivier, and B. M. Olivera. Dept. of Biology, University of Utah, Salt Lake City, UT 84112.

The conantokins are neurotoxins isolated from the venomous, fish-hunting marine snails of the genus *Conus* (McIntosh et al. (1984) J. Biol. Chem. 259:14343). These peptides, which induce either a sleep-like state or hyperactivity when injected i.c. into mice (≤2 weeks or ≥3 weeks, respectively), have been recently reported to target the N-methyl-D-aspartate(NMDA)-subtype of the glutamate receptor (Olivera et al. (1990) Science, In press). The conantokins contain 17-27 residues including at least four of the unusual amino acid γ-carboxyglutamate (Gla). Conantokin analogs have been chemically synthesized, radioiodinated, and crosslinked to NMDA receptor-enriched membranes (postsynaptic densities), with disuccinimidyl suberate. Following resolution by sodium dodecylsulfate polyacrylamide gel electrophoresis, autoradiography revealed primarily two bands with ~M_rs of 50 and 90 kd. The proteins of these bands were labeled specifically as determined by competition with excess cold ligand and are likely subunits of the same complex as evidenced by two-dimensional gel electrophoresis.

Whole rat brain membranes were detergent-extracted and subjected to affinity chromatography. Crosslinking analysis of the column retentate revealed enrichment of the 50 and 90 kd bands. Present results are consistent with the conantokin receptor being a multi-subunit ion channel complex. (Supported by GM22737.)

396.3

DISTRIBUTION OF NMDA RECEPTORS ON HIPPOCAMPAL NEURONS. O.T. Jones†,°J.F. McGurk, M.V.L. Bennett, °R.S. Zukin, #G. Collingridge, T. Benke†, K.J. Angelides†, †Department of Physiology, Baylor College of Medicine, Houston, TX, Department of Neuroscience, °Albert Einstein College of Medicine, Bronx, NY, #Department of Pharmacology, Bristol University, UK.

Excitatory amino acid transmission in the CNS depends critically on the activation of ligand-gated ion channel receptors classified by their preference for the agonists kainate, quisqualate and N-methyl-D-aspartate (NMDA). The NMDA receptor has aroused considerable interest due to its unique ability to translocate the second messenger calcium and its voltage dependent blockade by magnesium ions. To map the distribution of NMDA receptors at the neuronal cell surface we prepared several fluorescent and biotinylated analogs of Conantokin G a polypeptide isolated from the marine snail *Conus geographus*. The unmodified toxin blocked NMDA currents in *Xenopus* oocytes injected with rat brain mRNA and in hippocampal slices. NMDA receptors were mapped on cultured hippocampal neurons by colloidal gold decoration and circular dityndallism microscopy and their locations resolved by immunocytochemistry using antibodies to MAP-2 and synaptophysin, markers of dendrites and presynaptic terminals, respectively. Labeling was present on cell bodies and dendrites, was non-uniform, and was distinct from voltage-dependent calcium channel distribution labeled by ω-conotoxin. Using these fluorescent and biotinylated probes we are addressing how NMDA receptors are expressed, targeted and maintained during neuronal development. Visualization of NMDA receptors on live neurons will be a central feature in analysis of receptor distribution during synaptic plasticity. (Supported by the NIH, MRC, and Epilepsy Foundation of America.)

396.4

NMDA AND NON-NMDA RECEPTORS AGGREGATE AT SYNAPSES IN CULTURED CEREBRAL CORTICAL NEURONS. K.A. Jones and R.W. Baughman. Dept Neurobiology, Harvard Med School, Boston MA 02115.

Non-NMDA receptors contribute importantly to synaptic responses and aggregate on dendrites of neurons in cell culture. NMDA receptors, which play a key role in synaptic plasticity, are more difficult to localize because of the high affinity of the NMDA receptor for Glu. We combined electrophysiological and immunocytochemical mapping techniques to determine the relationship between receptor distribution and synapses. Whole-cell-patch recordings were made with neurons from rat visual cortex cultured for 5-34 days. Depolarizations were elicited by applying brief (0.5-1.5 ms) pulses of Glu with a fine iontophoretic pipet positioned within < 0.5 μm of the dendrite. Most cells had well defined sites of high sensitivity to Glu flanked by regions of low sensitivity. APV and CNQX were used to distinguish the contributions of the receptor subtypes at each site. Subsequently, the culture was processed for localization of the synapse-specific protein, SV2. In all of 24 cases, $SV2^+$ puncta were observed within 1 μm of a region of high Glu sensitivity. In 7 experiments, summarized below, complete analysis of receptor subtypes was obtained. (Data normalized to non-NMDA response at $SV2^+$ site.)

	$SV2^+$ regions	$SV2^-$ regions	Ratio $SV2^+$: $SV2^-$
non-NMDA	100%	12.1±8.4%	8.3 : 1
NMDA	41.9±21.2%	23.1±19.1%	1.8 : 1

The $SV2^+$:$SV2^-$ ratio for NMDA is in fact higher than 1.8 because of the high affinity of the NMDA receceptor for Glu. By measuring the drop off in response when the Glu pipet was moved orthogonal to the dendrite, we determined that to obtain a 90% decrement the pipet had to move 6 μm for non-NMDA and 20 μm for NMDA receptors. The distance between the $SV2^+$ and $SV2^-$ sites ranged 5-12 μm, indicating that we would have measured a significant NMDA response at the $SV2^-$ sites even if all of the NMDA receptors were localized at the $SV2^+$ sites. In conclusion, these data suggest that both non-NMDA and NMDA receptors are concentrated at synapses. (NIH EY03502)

396.5

IN VITRO AND EX VIVO BINDING OF ^{3}H-FTCP: A TOOL TO STUDY NMDA RECEPTOR COMPLEX IN VIVO. C. Ferrarese, A. Guidotti^, E. Costa^, K. Rice+, B. DeCosta+*, R.S. Miletich and G. Di Chiro*. Neuroimaging Sect., NINDS, Laboratory of Medicinal Chemistry,*NIDDK, NIH, Bethesda, MD and ^FGIN, Washington, D.C. 20007.

Fluorothienylcycloexypiperidine (FTCP), a derivative of TCP, includes a Fluorine atom and might be suitable for PET studies as a maker for NMDA sensitive glutamate receptors.

In vitro, the specific binding of ^{3}H-FTCP to rat and mice brain membranes was measured by subtracting the non specific binding in the presence of 10^{-5}M MK-801 or FTCP from the total binding. For *ex vivo* studies, 3 nmol/Kg of ^{3}H-FTCP (s.a. 14 μCi/nmol) was injected i.v. in mice and rats. The animals were sacrificed at different times and radioactive acetic acid extracts from various brain areas were counted before and after separation on sep-pak C18 cartridge. ^{3}H-FTCP is metabolized *in vivo* to hydrosoluble derivatives, with half lives of 7 min in mice and 15 min in rats. In rat brain regions the specific binding of ^{3}H-FTCP *ex vivo* was calculated 10 min after the i.v. injection by subtracting the amount of ^{3}H-FTCP in animals treated with 1 μM MK-801 from the amount of ^{3}H-FTCP present in the control animals.

^{3}H-FTCP specific binding is saturable *in vitro* and *ex vivo*, and the density of binding sites is highest in hippocampus, followed by cerebral cortex, and striatum; and lowest density is in cerebellum. Drugs acting on the PCP site of the NMDA receptor (MK-801, TCP, PCP) can displace ^{3}H-FTCP binding with nanomolar affinity; on the contrary, Di-o-Tolylguanidine (DTG), a specific ligand of sigma receptors, is active only at micromolar concentration. ^{3}H-FTCP binding is increased by about three fold in the presence of glutamate *in vitro*, and NMDA *in vivo*. However this facilitation fails to occur in cerebellum. These data suggest that FTCP is a suitable ligand for functional studies of NMDA receptors by PET scanning technique.

396.6

PHARMACOLOGICAL PROPERTIES OF KAINATE-ACTIVATED CHANNELS EXPRESSED FROM A RAT BRAIN cDNA. R. S. Zukin, R.S. Roginski, J. McGurk, M.V.L. Bennett. Dept. Neuroscience, Albert Einstein Coll. of Med., Bronx, N.Y. 10461.

The major excitatory neurotransmitter glutamate activates several different receptors, one of which can be identified by its responses to kainate. A complementary DNA encoding a kainate-activated channel was recently cloned (Hollmann et al., *Nature* 342:643-648, 1989), and the deduced 889-amino acid sequence indicates that it belongs to the large superfamily of ion channel forming receptors. We used the polymerase chain reaction to isolate an apparently identical clone from a rat brain cDNA library constructed in λZAP. *Xenopus* oocytes were injected with cRNA transcribed from this kainate receptor clone. After 48 hrs kainate was rapidly perfused over intact oocytes voltage-clamped at -60 mV. 500 μM kainate elicited a short latency, slowly rising (τ = 4 sec) inward current of up to 500 nA. This response was more slowly rising and falling than could be accounted for by perfusion delay (<0.5s). The dose-response curve for kainate showed a half-maximal effective concentration (EC_{50}) of 50 μM and a Hill coefficient of 1. Although kainate responses from rat brain mRNA had an apparent K_d of about 55 μM, the Hill coefficient was ca. 2.5 (Lerma et al., *PNAS* 86:2083-2087, 1989). The kainate responses were blocked by the selective kainate receptor antagonist CNQX (IC_{50} about 300 nM at 50 μM kainate); onset and reversal of block were rapid. No responses to NMDA or quisqualate were observed in oocytes injected with this cRNA. These findings indicate that the kainate receptor cDNA directs the expression of receptors similar, but not identical, to kainate receptors expressed from rat brain mRNA.

396.7

MOLECULAR CLONING OF A cDNA ENCODING A KAINIC ACID RECEPTOR SUBUNIT EXPRESSED IN HUMAN BRAIN AND RETINA. Tom Stormann, David Gdula, David Weiner, Allan Levey and Mark Brann. LMB/NINDS and Receptor Genetics, Inc., Bldg. 36, Rm. 3D-02, Bethesda, MD 20892

Recently, a cDNA encoding a subunit of a kainic acid receptor was isolated from rat brain by expression cloning (Hollman et. al, Nature, 1989). We have used oligonucleotide probes derived from the published rat nucleic acid sequence to screen human hippocampal and retinal cDNA libraries to isolate related excitatory amino acid receptor subunit cDNAs. Two 3 Kb cDNAs (one from the hippocampal library and one from the retinal library) which appear to encode the human homologue of the published rat cDNA have been isolated. The retinal cDNA encodes the complete coding sequence of a protein that shares 97% amino acid homology with the rat kainic acid receptor subunit. Oligonucleotide probes derived from the human sequence encoding the putative intracellular domain have been used to localize mRNA in the middle frontal gyrus, hippocampus, neostriatum, thalamus, midbrain, pons and cerebellum of human brain. The strongest hybridization signals were observed in the cerebellar cortex and the hippocampal formation, including the dentate gyrus and fields CA1 to CA4. Moderate signals were observed in cerebral cortex, the pontine nuclei and the substantia nigra. We are currently pursuing the detailed cellular mapping of the mRNA in human brain and retina as well as the functional expression of the receptor subunit in mammalian cells.

396.8

COOPERATING DNA BINDING PROTEINS AS NUCLEAR THIRD MESSENGERS IN PRIMARY CULTURES OF NEURONS. A.M. Szekely, R.E. Paulsen, E. Costa, and D.R. Grayson. FGIN, Georgetown University, Washington, D.C. 20007.

Coordinated changes in neuronal gene expression associated with NMDA sensitive glutamate receptor stimulation may be a key mechanism underlying long-term adaptive modifications. In primary cultures of rat cerebellar granule cells brief stimulation of this receptor resulted in a programmed induction of early response genes involving c-*fos*, c-*jun*, *jun*-B and *zif/268*. These genes encode nuclear proteins, which by interacting with regulatory DNA elements, function as third messengers bringing about coordinated regulation of target gene expression. Using gel shift assays and western blot analysis we show that following a brief glutamate pulse, the various Fos/Jun protein complexes (hetero and/or homodimers) display a biphasic binding to AP-1 DNA sequences. The composition of these complexes changes with time. The glutamate stimulation also results in an increased DNA binding activity of the zif/268 "zinc finger" protein to its consensus recognition sequence, which is structurally unrelated to the AP-1 site, and is found 5' upstream of several early response genes. This increased activity shows a delayed appearance compared to that of the AP-1 complex. These results, in light of our further data that different stimulus-specific patterns of early gene expression exist in these cells, prompt us to elucidate the mechanism whereby these nuclear third messengers interact and mediate a temporally flexible but precise response to transmitter receptor stimulation in neurons.

396.9

SEX STEROIDS ALTER N-METHYL-D-ASPARTATE (NMDA) RECEPTOR BINDING IN THE HIPPOCAMPUS. N.G. Weiland. Department of Physiology, University of Maryland, School of Medicine, Baltimore, MD 21201.

Sex steroids influence physiological processes mediated by the hippocampus. The hippocampus, a major component of the limbic system, plays a role in the regulation of feminine sexual and maternal behaviors as well as non-reproductive behaviors associated with learning and memory. NMDA, which activates one class of glutamate receptors, mediates some of the neurophysiological processes that underlie activity-dependent control of neuronal plasticity, long-term potentiation in the hippocampus, and learning and memory. Therefore, we examined whether estrogen (E) and progesterone (P) may affect these behaviors by altering NMDA receptors in the hippocampus (CA1). Female rats (3-4 months) were ovariectomized (OVX) for 9 days, OVX and E, or OVX and EP-treated. We measured the density of NMDA receptors by incubating 8μm brain sections in 150nM ^{3}H-glutamate and varying concentrations of NMDA (2-1000 μM) in Tris acetate buffer. Sections were rinsed briefly, dried, and exposed with ^{3}H-standards to LKB Ultrofilm. The films were quantitated on an image analysis system. E and EP increased the density of NMDA binding sites in the CA1 compared to OVX, whereas the Ki was unaffected. These results indicate that sex steroids may affect physiological processes mediated by the hippocampus by altering NMDA receptor densities. (Supported by American Federation of Aging Research)

396.10

THE HIGH AFFINITY NON-NMDA-COUPLED PCP-1 RECEPTOR IS REGULATED BY GUANINE NUCLEOTIDES. Y. Itzhak, I. Stein* and C. Kassim*. REPSCEND Labs. Dept. of Biochemistry & Mol. Biology, University of Miami School of Medicine, Miami, FL 33101.

We have previously reported that the potent phencyclidine analog, [^{3}H]-1-[1-(3-hydroxyphenyl) cyclohexyl] piperidine ([^{3}H]PCP-3-OH) labeles a high affinity binding site which is not coupled to the NMDA ion channel complex, sensitive to nM concentrations of PCP analogs and certain benzomorphans, but insensitive to haloperidol (Neurosc. Lett. 104: 314-319, 1989). Since this high affinity binding site is distinct from the PCP/NMDA receptor complex and the sigma/haloperdiol sensitive binding site, we have further attempted to characterize this site. Specific binding of [^{3}H]PCP-3-OH (0.6nM) to rat cerebrocortical membranes is reduced (20-65%) in a concentration dependent manner in the presence of Gpp(NH)p (0.05-1mM). However, binding of MK 801 to the PCP/NMDA receptor complex is not affected under the same conditions. In the absence of Gpp(NH)p homologous competition of [^{3}H]PCP-3-OH with unlabeled PCP-3-OH resulted in the best fit for a two site model, wherease in the presence of the nucleotide only one site was detected. Our studies also indicated that in the presence of Gpp(NH)p the PCP-1 binding site is not converted to the PCP/NMDA receptor complex, suggesting that the GTP-sensitive PCP-1 receptor site represent a distinct receptor domain.

396.11

SIGMA LIGANDS POTENTIATE NMDA-INDUCED HIPPOCAMPAL NEURON ACTIVATION. G. Debonnel, F.P. Monnet and C. de Montigny Neurobiological Psychiatry Unit, McGill University, Montréal, Québec, Canada.

We have previously reported that low doses of the sigma (σ) ligand DTG selectively potentiate NMDA-induced activation of hippocampal pyramidal neurons and that this effect of DTG is blocked by haloperidol, a high affinity σ ligand (*Neurosci. Abst.*, 15: 133.10, 1989). This suggested that a major role for σ sites might be to modulate the NMDA response. To further verify this hypothesis, we studied the effects of other selective σ ligands and of a structural analog of DTG devoid of affinity for σ sites, on neuronal responsiveness to excitatory amino acids in male Sprague-Dawley rats under urethane anaesthesia. Five-barrelled micropipettes were used for extracellular recording of CA_3 dorsal hippocampus pyramidal neurons and microiontophoresis of NMDA, quisqualate and kainate.

Similarly to DTG, low doses (1-10 μg/kg, i.v.) of (+)pentazocine, JO-1784 [(+) *N-cyclopropylmethyl-N-methyl-1,4-diphenyl-1-ethyl-but-3-en-1-ylamine*], BD-737 [(+)*-cis-N-methyl-N-[2-(3,4-dichlorophenyl) ethyl]-2-(1-pyrrolidinyl) cyclohexylamine*] and AdIpG [*1-(1-adamantyl)-3-(2-iodophenyl)*] markedly and selectively potentiated the excitatory effect of NMDA in a dose-dependent manner.

At low doses (1-10 μg/kg, i.v.), similarly to haloperidol, BMY-14802 and (+)3-PPP did not have any effect on NMDA-induced activation, but dose-dependently reversed the potentiation of NMDA by a low dose of DTG.

At doses up to 100 μg/kg i.v., 2-aminoperimidine hydrobromide, a structural analog of DTG without affinity for σ sites failed to produce an effect on NMDA-induced activation.

These data bring additionnal support to the notion that an important function of σ receptors might be to modulate the NMDA response. Furthermore, they suggest the existence of two classes of ligands: DTG-like compounds acting as "agonists" and haloperidol-like ligands as "antagonits" at σ sites.

396.12

NEUROPEPTIDE Y SELECTIVELY POTENTIATES THE NMDA RESPONSE. F.P. Monnet, G. Debonnel, T. Dennis and C. de Montigny. Neurobiological Psychiatry Unit, McGill University, Montréal, Québec, Canada and Institut de Recherche Jouveinal, Fresnes, France.

Recently, Roman et al. (*Eur. J. Pharmacol.*, 174: 301, 1989) reported that neuropeptide Y (NPY), the most abundant peptide in the mammalian brain, has high affinity for sigma (σ) binding sites. Several σ ligands, at low doses, selectively potentiate the NMDA-induced activation of hippocampal pyramidal neurons, whereas low doses of haloperidol, which also has high affinity for σ sites, reverse this potentiating effect (Monnet et al., *Neurosci. Abst.*, 15: 133.10, 1989; Debonnel et al., *this meeting*). The present electrophysiological experiments were undertaken to study the effect of microiontophoretic application of NPY on the neuronal activation induced by NMDA, quisqualate (QUIS) and kainate (KA).

Male Sprague-Dawley rats were anesthetized with urethane (1.25 g/kg, i.p.). Five-barrelled micropipettes were used for extracellular recording of CA_3 dorsal hippocampus pyramidal neurons and microiontophoresis of NPY (0.1 mM in 150 mM NaCl and 0.1% BSA, pH: 8), NMDA (10 mM in 200 mM NaCl, pH: 8), QUIS (1.5 mM in 400 mM NaCl, pH: 8), KA (1 mM in 400 mM NaCl, pH: 8) and haloperidol (0.2 mM in 200 mM NaCl, pH: 4).

Prolonged microiontophoretic application of NPY increased by two-fold the neuronal response to NMDA with a latency of 15-30 min, without altering those to QUIS and KA. This effect of NPY was reversed by the systemic administration of haloperidol (20 μg/kg, i.v.). Microiontophoretic application of haloperidol also reversed the NPY-induced potentiation of the NMDA response.

These data suggest that a major role of NPY might be to modulate the function of NMDA receptors. The reversal of the effect of NPY by haloperidol suggests that it might be due to the affinity of NPY for σ sites.

397.1

FACTORS INVOLVED IN THE REGULATION OF β_1- AND β_2-ADRENERGIC RECEPTOR mRNA IN RAT C_6 GLIOMA CELLS. C. Hough and D.-M. Chuang. Biological Psychiatry Branch, NIMH, Bethesda MD 20892

Exposure of C_6 cells to β-adrenergic agonist, isoproterenol, leads to down-regulation of the mRNA species for both β_1- and β_2-adrenergic receptors (β-AR) within 4 hours. The mechanism of down-regulation for the two receptor subtypes differs, however, in that β_2-AR mRNA simply decays to 20% of pretreatment levels while β_1-AR mRNA is transiently up-regulated before decaying to 40% of pretreatment levels. Both modes of regulation induced by isoproterenol are inhibited by β-AR antagonists, alprenolol, propranolol, betaxolol, and ICI 118 551. Alone, these antagonists can have effects on β-AR mRNA levels similar, albeit less potent, to those of isoproterenol. Both modes of regulation can be mimicked at slower rates by agents that can raise intracellular cAMP, forskolin, cholera toxin, and 8-Br cAMP. In the presence of transcription inhibitor, actinomycin D (10 μg/ml), both β_1- and β_2-AR mRNA decay at approximately the same rate ($t_{1/2}$=30 min), which is not significantly changed by isoproterenol treatment. In the presence of protein synthesis inhibitor, cycloheximide (10 μg/ml), β_1- and β_2-AR mRNA are dramatically stabilized, even in the absence of transcription. These results suggest that regulation of β-AR mRNA occurs at the levels of transcription and translation.

397.2

AN EXON WITH A STOP CODON IS SELECTIVELY SPLICED INTO EMBRYONIC TRANSCRIPTS OF THE GLUTAMIC ACID DECARBOXYLASE GENE. R.J. Wyborski, R.W. Bond and D.I. Gottlieb, Dept. of Anatomy and Neurobiology, Washington Univ. Sch. of Med. St. Louis, Mo 63110

In the adult rat brain the gene for Glutamic acid decarboxylase (GAD) is expressed as a 3.7 kb transcript coding for a 67 kDa protein. An alternative form of the GAD transcript was detected in fetal brain by nuclease protection assay. (Bond et. al. PNAS 85:3231, 1988) The structure of the alternative form is reported here. A segment of the GAD mRNA from fetal brain was reverse transcribed and amplified by PCR. The sequence consisted of the known adult structure with an additional 86 bp exon. Conceptual translation of the novel structure showed a stop codon in frame with the open reading frame of the message and predicts a truncated protein product of 25 kDa. The stop codon is 5' to the pyridoxal phosphate cofactor binding site; therefore the predicted truncated protein cannot function as a decarboxylase. Genomic clones with the GAD gene have been isolated. One of these clones contains the 86 bp exon flanked by intronic sequences. To determine the level of expression of the embryonic exon, protection assays were done utilizing a probe containing the embryonic exon. These assays show that the embryonic form is present throughout the embryonic brain but not in the adult. These data suggest that the appearance of functional GAD requires two types of control. First, transcription must be initiated. Next, the splicing machinery must be regulated so as to eliminate the stop codon containing exon from the mRNA so as to produce functional GAD.

397.3

TWO GENES ENCODE DISTINCT GLUTAMATE DECARBOXYLASES WITH DIFFERENT RESPONSES TO PYRIDOXAL PHOSPHATE. M.G. Erlander, N.J.K. Tillakaratne, S. Feldblum, N. Patel, and A.J. Tobin, Department of Biology, UCLA, Los Angeles, California 90024

Gamma-aminobutyric acid (GABA) is unmatched by any other known inhibitory neurotransmitter in its almost ubiquitous distribution in vertebrate brain. In adult brain, the synthesis of GABA depends on the activity of glutamic acid decarboxylase (GAD; E.C.4.1.1.15). The mammalian brain contains at least two forms of GAD, which differ from each other in size and in interaction with the cofactor pyridoxal-5'-phosphate (PLP). This report answers a fundamental question posed since the early 1970s: is the observed heterogeneity the result of more than one gene encoding mammalian GAD? We demonstrate here the existence of two GAD genes which encode proteins that differ in molecular size, response to PLP, and subcellular location. Furthermore, the two GAD mRNAs are present in the same neuronal types of the rat cerebellum, but at different levels. We suggest that the different properties of these two genes allow greater flexibility in regulating GABA synthesis. In addition, convulsive doses of PLP antagonists in-vivo may preferentially inhibit GAD_{65}, encoded by the new cDNA we describe in this report. Supported by NS22256 and 20256 to AJT.

397.4

PARALLEL REGULATION OF mRNAs FOR DOPAMINE β-HYDROXYLASE AND TYROSINE HYDROXYLASE BY DEXAMETHASONE, cAMP AND GROWTH FACTORS IN PC12 CELLS. A. McMahon*, H. Badoyannis, S.C. Sharma and E.L. Sabban, New York Med. Coll., Valhalla, NY 10595.

Dopamine β-hydroxylase (DBH), which catalyzes the conversion of dopamine to norepinephrine, is regulated by various hormonal and neuronal factors. To study the molecular mechanism of this regulation, we examined the effects of glucocorticoids, cAMP and growth factors on DBH mRNA in PC12 pheochromocytoma cells.

Dexamethasone (1 mM) increased both the 2.7 and 2.5 Kb mRNAs for DBH about 4-fold. This increase was apparent by 7 hrs and maximal at 2 days of treatment. Treatment with 8-bromo-cAMP (1 μM), or forskolin (10 μM) also elevated both mRNAs for DBH, with maximal induction of 3-fold by 8 hrs. DBH mRNA remained elevated for 1 day, but with longer exposures decreased to below control levels. Combined treatment with 8-bromo cAMP and dexamethasone gave results similar to 8-bromo cAMP alone. The mRNA levels for tyrosine hydroxylase (TH) essentially paralled the induction of DBH mRNA with either dexamethasone or cyclic AMP. Nerve growth factor, in contrast, decreased both DBH and TH mRNA levels.

The results of this study demonstrate that DBH mRNA is subject to regulation by several physiologically important factors. Genomic clones to rat DBH have been isolated to begin to identify regulatory elements.

397.5

COMBINED NICOTINIC AND MUSCARINIC REGULATION OF CO-LOCALIZED ADRENAL TRANSMITTER GENES: PREPROENKEPHALIN (ppENK) AND TYROSINE HYDROXYLASE (TH). J.D. DeCristofaro, G. Weisinger and E.F. La Gamma, Dept. of Pediatrics, SUNY at Stony Brook, NY 11794-8111.

Combined nicotinic and muscarinic stimulation results in a greater than additive rise in steady state ppENK mRNA, enkephalin prohormone and peptide (Neuroscience 35:203, 1990; Neuroscience Abs 142.4, 1989). The rise in ppENK is 50-fold after 2 days and 100-fold after 4 days of treatment. Steady state TH mRNA peaks at 2 days and plateaus at 10-fold over baseline. Both ppENK mRNA and TH mRNA levels return to baseline within 1 week following the 4 day treatments. This suggests independent regulation of these co-localized messages. To determine the contribution of transcriptional initiation on this rise and fall in steady state ppENK mRNA, S1 and primer extension analysis were performed. The greatest rise in ppENK RNA initiation occurred after 2 days of combined cholinergic treatment. By a week after completion of 4 day treatments, three of the four start sites identified by primer extension returned to baseline. These data suggest that cholinergic induced initiation of ppENK RNA transcripts contributes to the rise in steady state mRNA. Moreover, stabilization of steady state ppENK mRNA may also play a role since initiation decreases after 2 days of treatment, but RNA levels continue to rise up to 4 days. Supported by NSF #BNS8719872.

397.6

TYROSINE HYDROXYLASE (TH) GENE EXPRESSION IN BOVINE ADRENAL CHROMAFFIN CELLS: REGULATION BY CHOLINERGIC RECEPTOR STIMULATION. M. Olasmaa, M. Memo, D. Grayson, A. Guidotti and E. Costa. FGIN, Georgetown University, Washington, D.C. 20007.

Cyclic AMP-mediated TH gene expression in adrenal chromaffin (AC) cells is transsynaptically regulated by neurotransmitters released from the splanchnic nerve terminals (Biochem.Pharmacol. 26 (1977) 817-823). Acetylcholine (ACh) has been shown to be a major transmitter released from splanchnic nerve. Primary cultures (5-7 days in vitro) of bovine AC cells were utilized as a model system. Exposure of the AC cells to 0.1-1.0 mM carbachol slightly (ca. 20%) increased the TH-V_{max} when measured 48hrs after the beginning of the treatment. However, if carbachol was applied together with HL-725 (Trequinsin, 10^{-5}M), a type II phosphodiesterase (PdE) inhibitor (Hoechst-Roussel Pharm., Inc.), the increase was enhanced (ca. 70%). The facilitatory effect of HL-725 confirmed the involvement of cAMP in the transduction regulating the level of TH-V_{max} (Mol.Pharmacol. 16 (1979) 865-876). Since in splanchnic nerve terminals ACh coexists with VIP, which is known to utilize cAMP for its signal transduction, the possibility that VIP functions as a co-transmitter with ACh in the transsynaptic induction of TH was investigated. VIP (10^{-7}-10^{-5} M) produced a dose and time dependent increase of TH-V_{max}, which in the presence of HL-725 was shifted to the left, indicating an involvement of cAMP as a second messenger in this response. When AC cells were exposed to 1μM VIP, without HL-725, a slight increase in TH-V_{max} was observed. However, this low dose facilitated the action of carbachol. The change in TH mRNA content elicited by 10 μM VIP together with HL-725 was investigated following amplification by polymerase chain reaction. The combination VIP and HL-725 increased the TH mRNA content in a time-dependent fashion.

397.7

TIME DEPENDENT REGULATION OF TYROSINE HYDROXYLASE mRNA IN S. NIGRA DOPAMINERGIC NEURONS FOLLOWING 6-OHDA LESION. G.M. Pasinetti, J.F. Reinhard, A.B. Kelly, D.G. Morgan and C.E. Finch. Andrus Gerontology Center and Dept. of Biological Sciences, Univ. of Southern California, Los Angeles, CA, 90089-0191. The Wellcome Research Laboratories, Research Triangle Park, NC 27709.

Long term nigral 6-hydroxydopamine (6-OHDA) lesions induce atrophic changes in the remaining s. nigra dopaminergic (DAergic, tyrosine hydroxylase (TH) immunopositive) neurons, including >50% loss of TH-mRNA concentration per neuron [Pasinetti et al., Mol. Brain Res. 5:203 (1989)]. We extended these findings by examining the time course of changes in the remaining s. nigra DAergic neurons at 2,21,90 and 270 days following nigral 6-OHDA lesions. TH catalytic activity, TH polypeptide content, dopamine (DA) and DA catabolites (HVA and DOPAC) at the striatal terminals, were measured to resolve multiple levels of DAergic regulation in response to nigral lesioning. Nigral atrophic changes began as early as 90 days post-lesion as indicated by a reduction of nigral DAergic perikaryal area (-20%) and decreased TH-mRNA concentration (-30%) per neuron. The decreased TH-mRNA concentration at 270 days confirms previous results. In contrast to these atrophic nigral changes, compensatory changes were observed in striatum. At 21 days post-lesion, the striatal DA catabolite, HVA, was reduced slightly less than TH catalytic activity, which suggests increased (+30%) striatal DA release and utilization. Because the loss of striatal TH polypeptide content was smaller than the loss of DAergic neurons at all times after nigral 6-OHDA lesions, it follows that the surviving s. nigra DAergic neurons compensated by increased production of TH protein. Thus, the surviving neurons become more efficient in utilizing the available TH-mRNA with regard to the final product, striatal TH polypeptide. (Supported by National Parkinson Foundation and United Parkinson Foundation to CEF and GMP and by John D. and Katherine T. MacArthur Foundation Research Program on Successful Aging to CEF. DGM is an Established Investigator of Amer. Heart Assn.).

397.8

LOCAL PROTEIN SYNTHESIS WITHIN ISOLATED GROWTH CONES OF CULTURED SNAIL NEURONS. Lauren Davis and Stanley B. Kater. The Program in Neuronal Growth and Development and the Department of Anatomy and Neurobiology, Colorado State University, Fort Collins, CO 80523.

The neuronal growth cone is a highly specialized region at the tip of growing neurites that contains a complement of organelles that is different from the rest of the neurite and perikaryon, and an intracellular calcium concentration that is apparently independently regulated. It has been suggested that growth cones may function independently from the rest of the neuron and, in fact, the behavior of isolated growth cones is indistinguishable from that of intact for at least 48 hours. Further autonomy would be conferred by the ability of such an independent system to locally synthesize proteins. To explore the possibility that proteins are locally synthesized in growth cones, cultures of Helisoma neurons were prepared and the growth cones of 1-2 neurites per cell were transected. Two to 6 hours following transection, cultures were pulse-labelled in 3H-leucine for 10 minutes and then fixed and processed for autoradiography. Autoradiographs demonstrated that radiolabeling over isolated growth cones was comparable to labeling over growth cones and distal neurites whose cytoplasm was continuous with the rest of the cell. Because snail neurons are impermeable to most conventional protein synthesis inhibitors, we employed DMSO (8%), a reversible inhibitor of ribosomal protein synthesis, at a concentration that pulse-treated neurons survived and continued to grow for at least 2 days. Labeling was greatly reduced or eliminated over neurons, as well as isolated growth cones, in DMSO treated cultures; a control that suggests that 3H-leucine is indeed incorporated into newly synthesized proteins during translation. These results suggest that proteins are locally synthesized in isolated neuronal growth cones. This may indicate that the expression of the genome can be regulated independently by individual subcellular regions. Thus, the complement of protein components within a growth cone might be modified in response to local stimuli. Supported by NIH NS08445.

397.9

ISOLATION OF NOVEL DEVELOPMENTALLY REGULATED GENES FROM EMBRYONIC MOUSE TELENCEPHALON USING SUBTRACTIVE HYBRIDIZATION. J.L.R. Rubenstein, M.H. Porteus*, A.E.J. Brice*, R.D. Ciaranello and T.B. Usdin. Laboratory of Developmental Neurochemistry, S253, Stanford Univ. Sch. of Med., Stanford, CA 94305.

We are studying the genetic mechanisms which regulate development of the mammalian forebrain. To this end, we have devised an efficient method of performing subtractive hybridization between directional cDNA libraries in single-stranded phagemid vectors. We have performed subtractions in which the driver was cloned cDNA from adult mouse telencephalons, and the target was cloned cDNA from embryonic day 15 telencephalons. We obtained a 70-fold subtraction. We have performed northern analysis of five of the clones; four of them are preferentially (20-1000 fold) expressed in the embryonic telencephalon. The partial DNA sequences of these clones are not found in Genebank. One of the clones has strong homology to the helix III region of the antennapedia homeodomain. We are presently further characterizing the cDNAs by *in situ* hybridization.

397.10

EFFICIENT UPTAKE AND EXPRESSION OF FOREIGN DNA BY CNS STEM CELLS D.I.Gottlieb, G.T.Coker III, J. Ho*, M. Moffat* and K.L. O'Malley, Dept of Anatomy and Neurobiology, Washington University Sch of Med., St. Louis Mo. 63110

By introducing foreign DNA into eukaryotic cells, the mechanisms underlying cell specific and hormonally regulated gene expression can be analyzed. We have extended this approach to cells of the early chick embryonic brain. Suspensions of viable E5 tectal cells were prepared by trypsinization. Plasmid DNA was introduced into these cells via electroporation. Plasmids containing the β-galactosidase reporter gene driven by the Rous sarcoma virus (RSV) promoter were efficiently expressed. 44 hours after electroporation 22% of the cells were positive for the β-galactosidase marker. When cells were cultured on laminin for 4 days after electroporation many axon bearing cells were stained. Therefore some of the cells which take up and express foreign DNA can differentiate into neurons. In other experiments a promoter containing 6 cAMP response elements driving a CAT reporter gene was used. Transfected cells responded to added 8 bromo cAMP with a 40 fold increase in CAT activity. We conclude that some cells in the E5 tectum transcribe CREs in a cAMP dependent manner. Preliminary data with an RSV-luciferase construct were obtained. Levels of luciferase expression were sufficiently high to suggest that this approach will be applicable to a wide range of endogenous cellular promoters.

SENSORY SYSTEMS—VISUAL PSYCHOPHYSICS AND BEHAVIOR II

398.1

TOWARDS A NEUROBIOLOGICAL THEORY OF VISUAL AWARENESS. C. Koch and F. Crick, CNS Program 216-76, Caltech, Pasadena, CA 91125 and Salk Institute, La Jolla, CA 92037.

We propose a neurobiological framework to study one particular form of consciousness, visual awareness. We postulate one form of awareness linked to the serial, attentional mechanism studied by Julesz, Treisman and others. This working awareness (WA) solves the binding problem, i.e. rapidly and transiently signalling the combination of perceptual features. Such a mechanism is required to bind together all those neurons actively responding to different aspects of the perceived object in a coherent way. Furthermore, objects for which the binding problems has been solved are placed into short-term (i.e. several sec) memory. In order to explain the perceptual richness of our environment, we postulate a second, very fleeting form of awareness. This form is associated with iconic memory (< 500 msec), has a very large capacity and does not solve the binding problem.

One possible implementation of WA could involve the semi-synchronous neuronal oscillations in the 35-70 Hz range observed in visual and olfactory cortex (Freeman; Gray and Singer; Eckhorn *et al,*). Once visual attention has selected the most "interesting" location (using a saliency map), feedback pathways synchronize the oscillations at the corresponding locations in V1, V4, MT etc. Thus, the neuronal expression of attention is phase- and frequency-locked 40 Hz oscillations. These phase-locked oscillations activate short-term memory. Processes, such as priming and subliminal perception, which by-pass these oscillations will not be "consciously perceived".

398.2

COHERENT OSCILLATIONS IN A POPULATION-BASED MODEL: THEIR ROLE IN VISUAL PERCEPTION. O. Sporns*, G. Tononi*, and G.M. Edelman, The Neurosciences Institute, 1230 York Ave., New York, NY 10021.

Orientation-selective neurons in the cat visual cortex show stimulus-dependent oscillatory firing that can be coherent both locally as well as over long distances in the cortex. To investigate (1) how such neuronal oscillations might be generated, (2) how spatial coherency of oscillations arises and (3) what the functional significance of coherent oscillations might be, we present a computer model of two distinct visual cortical areas, roughly based on cortical areas V1 and MT (PNAS 86, 7265). Each area contains a number of neuronal groups composed of cells selective for the orientation and motion of line segments. Neuronal groups interact via reciprocal excitatory connections. Oscillatory firing is produced by cooperative interactions within each group. The intrinsic frequency of individual neuronal groups is highly variable (due to their variable structure) and shifts considerably within brief time periods. Phase coherency of distant groups arises from the reciprocal exchange of signals in reentrant connections. The magnitude of cross-correlations is sensitive to both number and strength of reentrant connections. Variations in intrinsic frequency prevent accidental phase-locking of coactive but unrelated groups. Coherent firing in the model depends critically on global stimulus properties such as spatial continuity and pattern motion. We present several examples of how coherent oscillatory activity can be used to transiently link features of a stimulus analyzed in segregated parts of the cortex. This mechanism might allow the distinction of stimuli from a background or, in general, the segmentation of a complex visual scene into distinct objects.

(Supported by the Neurosciences Research Foundation)

398.3

VISUAL NEURON RELIABILITY IN CONVEYING DIMENSIONAL INFORMATION AS A FUNCTION OF STIMULUS DURATION OR CONTRAST. Ehud Zohary * and Shaul Hochstein, Neurobiology Department, Institute of Life Sciences, Hebrew University, Jerusalem, Israel.

There is considerable recent interest in the neuronal source of the limited accuracy with which subjects perform visual tasks under contrast and/or time constraints. One approach to this question is the use of Signal Detection Theory and the comparison of the reliability of the responses of single neurons with the overall performance of the visual system in psychophysical tasks (reviewed by R.D. Freeman's special lecture at this meeting). As a special application of this technique we have compared the dependence of neuronal reliability and that of task performance on available processing time (Soc. Neurosci. 1989). Signal Detection Theory gives a good estimate of the neuron's ability to discriminate between two stimuli. However, it does not give a measure of the reliability with which the neuron's response signals the value of the stimulus over an entire range of a variable dimension. Information Theory provides a measure for this quantity (Werner and Mountcastle, 1965; Tolhurst, 1989). We now use this Information Theory approach to compare the information conveyed by the single neuron and psychophysical task performance. We studied the responses of macaque V1 neurons in the alert behaving preparation, as a function of stimulus orientation, spatial frequency, and motion direction and velocity. We compared the response information content with that inherent in the task performance. The information content concerning each of these dimensions is a function of the contrast and duration of the stimulus. We report these dependences for the single neuron, and compare these dependences with the parallel psychophysical measures.

398.4

DOES MOTION PERCEPTION DEPEND ON THE MAGNOCELLULAR PATHWAY? William H. Merigan, Carey Byrne, and John Maunsell, University of Rochester, Rochester, N.Y. 14642.

There is some evidence that visual signals relayed through magnocellular layers of the lateral geniculate (M pathway) may be particularly important for motion processing. Anatomical and physiological data show that this pathway provides the major input to the cortical "motion" pathway, which includes areas MT and MST. In addition, a recent lesion study has shown that damage to the M pathway disrupted motion detection, whereas damage to the P pathway had no such effect (Schiller et. al., 1990).

Ibotenic acid lesions were placed in magnocellular layer 1 of the lateral geniculate of macaques and two aspects of motion perception were tested in portions of the visual field of the contralateral eye that corresponded to the lesions. Velocity difference thresholds were measured with patches of drifting sinusoidal gratings. In addition, contrast sensitivity was measured for the detection of drifting grating patches, and for the discrimination of their direction of motion.

Performance on all measures was degraded by magnocellular lesions, and this was more pronounced at high speed (20 deg/sec) than at low speed (1 deg/sec). However, the observed changes did not suggest a deficit specific to motion perception. While contrast thresholds for discrimination of the direction of motion were raised by lesions, especially at higher speeds, similar changes were seen in grating detection thresholds. Velocity difference thresholds were greatly elevated in the vicinity of M pathway lesions, but it was possible to reduce or eliminate this difference by raising the contrast of the test stimuli. These results suggest that cortical motion mechanisms do not rely exclusively on input from the M pathway, but that it is important to visual detection at high speeds.

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398.5

CORTICAL DYNAMICS OF VISUAL MOTION PERCEPTION: SHORT- AND LONG-RANGE APPARENT MOTION. Michael E. Rudd and Stephen Grossberg, Center for Adaptive Systems, Boston University, 111 Cummington Street, Boston, MA 02215

Further development of a neural network model of motion perception by visual cortex (Grossberg and Rudd, 1989) is presented. The model clarifies computational differences between parallel cortical streams for perception of static form ($V1 \rightarrow V2 \rightarrow V4$) and moving form ($V1 \rightarrow MT, V2 \rightarrow MT$). Properties of the model are tested by simulation of psychophysical data concerning two-flash and Ternus apparent motion displays, as well as percepts of split motion, reverse motion, gamma motion, and interactions between continuous motion and binary color percepts. The model explains the approximate space-time separability of the motion strength function as well as deviations from space-time separability at threshold. The model clarifies the dependence of the maximum extent of short-range motion on element scale, and the different properties of short-range and long-range motion vis-a-vis interframe changes in stimulus direction-of-contrast. It specifies how sustained and transient channels cooperate and compete in successive processing stages to generate motion signals that are independent of direction-of-contrast; and how preprocessing of motion signals by a Motion Oriented Contrast (MOC) Filter is joined to long-range cooperative-competitive feedback mechanisms, called a CC Loop, to control phenomena such as induced motion and motion capture.

REFERENCES: Grossberg, S. and Rudd, M.E. (1989). *Neural Networks*, **2**, 421.

398.6

OBJECT REPRESENTATION THROUGH SURFACE INTERPOLATION IN STRUCTURE FROM MOTION

S. Treue, R. A. Andersen, H. Ando, E. C. Hildreth.
Dept. of Brain and Cognitive Sciences, M.I.T., Cambridge, MA 02139

Investigations in depth perception using disparity information have demonstrated surface interpolation between feature elements. Current computational algorithms of structure from motion (SFM) do not perform such an operation. Here we present strong evidence for surface interpolation in SFM and in the following abstract demonstrate how such a mechanism can be incorporated into current computational algorithms.

Using the parallel projection of randomly positioned dots on a rotating transparent cylinder, we impose a desynchronized oscillatory motion on the individual dots. Although such a stimulus does not have a rigid physical correlate, subjects perceive a rigid cylinder in smooth rotation. This percept can only be explained if the visual system represents the observed object not as a collection of individual elements but as two surfaces moving in opposite directions. A given point contributes information to one of the surfaces depending on its direction.

The basis for this segmentation of surfaces might be direction selective cells in V1 which act as directional filters ignoring motion in the non-preferred direction (Erickson et al. Neurosci. Abstr., 1989). In a population of such cells our stimulus would activate two distinct groups of cells and an oscillating dot would activate alternately either one or the other group of cells.

398.7

RECOVERING 3-D STRUCTURE FROM MOTION WITH SURFACE RECONSTRUCTION.

H. Ando, E. C. Hildreth, S. Treue, R. A. Andersen.
Dept. of Brain & Cognitive Sciences, MIT, Cambridge, MA 02139.

We present a model for the human recovery of 3-D structure from motion that combines an extension of Ullman's incremental rigidity scheme with a surface interpolation process that reconstructs full 3-D surfaces from sparse depth information. The input to this algorithm consists of either the velocities or displacements of moving points in the image. Using this information, the algorithm sequentially estimates a new 3-D structure by minimizing the overall deviation from rigidity. As 3-D structure is derived at the locations of the moving points, a smooth surface is filled in between these locations. Since what is preserved between views is the reconstructed surface, individual image features may appear and disappear without destroying the recovered structure.

This model allows multiple surfaces to be represented simultaneously in order to cope with transparency, facilitates the use of constraints on surface shape from object boundaries, and groups points on different surfaces based on their 2-D image motion. Thus the model can account for experimental observations by Husain et al. (Neural Comp. 1:324-333, 1989) and Treue et al. (preceding abstract) regarding the ability of human subjects to interpret 3-D structure in displays with limited lifetimes. We show that this model can account for many other experimental observations such as general degradation of performance with fewer points, as well as the interaction between multiple transparent surfaces in motion suggested by Ramachandran et al. (Perc. & Psychophys. 44:330-393, 1989).

398.8

LESIONS OF AREA 17 IN THE CAT REDUCE SENSITIVITY TO HIGH BUT NOT LOW SPATIAL FREQUENCY TARGETS. *Tatiana Pasternak, Iwona Zurawska, and John H. R. Maunsell.* Depts of Neurobiology and Anatomy, Physiology, and Center for Visual Science, University of Rochester, Rochester, NY 14627

We have recently shown that ibotenic lesions of area 18 severely reduce the sensitivity of cats to drifting low spatial frequency gratings but leave acuity intact (Pasternak et al., 1989). This result is consistent with the physiological properties of neurons in area 18, which on average prefer targets of spatial frequencies three times lower than neurons in area 17. The intact acuity following lesions of area 18, and the high spatial frequency preferences of area 17 neurons suggest that the detection of high spatial frequencies may depend upon neurons in area 17. To assess the contribution of area 17 to spatial vision we placed punctate unilateral ibotenic acid lesion in a physiologically identified portion of area 17. Each lesion was centered at an eccentricity of about 8° along the horizontal meridian of the left visual field. We measured the detectability of various spatiotemporal targets placed within the ablated portion of the visual field representations, while monitoring eye position with a scleral search coil. The cats were required to maintain fixation on a laser spot and respond to the presence or the absence of the grating by pressing a right or left pedal. We found a loss (30%) of visual acuity for targets placed within the lesioned part of the visual field. Furthermore, contrast sensitivity for stationary gratings of intermediate and higher spatial frequencies was reduced by a about a factor of 2. On the other hand, contrast sensitivity was normal at low spatial frequencies (0.28 c/deg). The results were similar for gratings drifting at 4.5 Hz. This result, in conjunction with previously reported deficits following lesions of area 18, demonstrates a high degree of functional segregation at early stages of cortical processing, with area 17 playing an important role in spatial resolution, while area 18 contributes to the detection of low spatial frequencies.

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398.9

EXTRASTRIATE LESIONS AND ILLUSORY CONTOUR ORIENTATION DISCRIMINATION IN THE CAT. P. De Weerd*, J.M. Sprague, E. Vandenbussche* and G.A. Orban. Lab. Neuro-en Psychofysiologie, K.U. Leuven, B-3000 Leuven, Belgium.

We have investigated the effect of large extrastriate lesions (Vandenbussche et al., Soc. Neurosci. Abstr. 15: 1255, 1989) on orientation discrimination (OD) in two cats (52 and 54), trained in OD of a bar and of two types of illusory contours. The length of illusory contours and bar was 12 deg. The contours used were Gap Illusory Contour (GIC) - 7 semicircles separated by 1.2 degree wide gap - and Phase Shifted Illusory Contour (PSIC) - 4 semicircles shifted in phase along the contour. 73.5% correct Just Noticeable Differences (JNDs) were determined for each contour-type in an interleaved way, presenting one contour-type per daily session. Although we have no histological control of the lesion yet, it was intended to destroy areas 19, 20 and 21, and medial bank of the Lateral Suprasylvian Sulcus. The two cats behaved very similarly during pre- and postoperative testing. Average preoperative JNDs were 5.8, 11.1 and 15.9 degrees for bar, GIC, and PSIC respectively. During the first postoperative month, no reliable thresholds could be measured for the PSIC, whereas thresholds for the bar and the GIC were 11.8 and 33.4 degrees, respectively. After three months JNDs stabilized at 7.5, 23.0 and 38.3 degrees. These results suggest that extrastriate areas are more critical for OD of illusory contours than for OD of bars.

398.10

CORTICAL AREAS MEDIATING RECOVERY FOLLOWING EARLY PERIODS OF MONOCULAR DEPRIVATION IN KITTENS. C.J. Beaver* and D.E. Mitchell. Dept. of Psychology, Dalhousie University. Halifax, Nova Scotia. B3H 4J1.

Despite the severity of the visual deficit produced by monocular deprivation, substantial recovery is possible if normal visual input is restored to the deprived eye sufficiently early, particularly if at the same time the initially non-deprived eye is occluded. During this period of reverse occlusion (RO) the initially deprived eye can recover to nearly normal acuity levels. Surprisingly, following termination of RO the visual acuity of the formerly deprived eye drops rapidly to less than half the values obtained during the period of RO, while at the same time the vision of the other eye gradually improves to almost normal levels. In order to determine whether these reciprocal changes are governed by areas 17/18, we examined the effect of bilateral ablation of these areas. Six kittens were monocularly deprived until 6 wks. of age, at which time they were reverse occluded for a further 6 wks. and then allowed a period of binocular vision. Following the period of binocular vision, bilateral lesions of areas 17/18 were made and after several months of recovery the visual acuities of both eyes were again measured. In normal animals, lesions of areas 17/18 reduce the acuity of both eyes by approximately a factor of two. If the reciprocal changes in vision observed after RO result from similar processes in all cortical visual areas, then a proportional drop in the visual acuity of both eyes would be expected. Rather than a proportional decrease, the acuities of the two eyes were very similar (almost half normal values). This result strongly suggests that the mechanism governing the rapid changes following reverse occlusion is located in areas 17/18.

398.11

LONGITUDINAL DEVELOPMENT OF EYE ALIGNMENT IN KITTENS WITH TWO TYPES OF SURGICALLY INDUCED STRABISMUS: RELATIONSHIP TO EXTRASTRIATE CORTICAL BINOCULARITY by Ruxandra Sireteanu,* Wolf Singer,* Maria Fronius,* Joachim Greuel and Johannes Best.* *Max-Planck-Institute for Brain Research, Frankfurt/Main, FRG*

Interocular alignment was assessed by corneal light reflex photography in 15 normal and 24 strabismic kittens. Nineteen strabismic and 5 normal kittens were followed longitudinally from 12 days to about 6 months of age. Strabismus was induced at 3-4 weeks of age, either by cutting one extraocular muscle (tenotomy) or by reinserting the muscle at another position on the ocular globe (recession).

Four out of the 6 tenotomized cats and 5 out of the 11 recessed cats conserved their post-operative ocular deviation throughout the testing period ("large-angle strabismics"). Two tenotomized and 6 recessed cats showed a transient deviation for 1-2 weeks after surgery, after which the interocular alignment stabilized to values found in normal cats ("microstrabismic" cats).

All cats showed a clear breakdown of binocularity in area 17. In the postero-medial lateral suprasylvian area (PLMS), binocularity was markedly reduced for the large-angle strabismics, but much less so for the microstrabismic cats.

The difference might be due to the coarse grain and poor retinotopic organization of visual receptive fields in the lateral suprasylvian area.

398.12

SIGNAL AND MESSAGE IN DIGITIZED SMILES. C.M. Leonard, Dept. of Neuroscience, Univ. Fl. College of Medicine, Gainesville, FL 32610.

One of the most complex information processing tasks that the brain can perform is the decoding of social communication signals such as facial expressions. There are few methods presently available for measuring information content in these kinetic displays. This abstract describes a technique for measuring the information generated by mouth movements in digitized human smiles. Facial movements were measured by digitally subtracting adjacent frames from eight 400-msec taped vignettes (12 frames) and calculating the variance of the distribution of pixel values in the subtracted images. The eyes were held constant in all frames. Twelve observers were asked to identify the social messages conveyed by the individual images in each vignette. The total number of positive messages attributed to each smile correlated .74 (Spearman rho, $p < .05$) with its cumulative variance. In five of the smiles, the perception of positive messages increased disproportionately during 100-msec periods of high variance associated with a deepening dimple on the left side.

In order to measure the relation between changes in signal and perceived message for individual observers, a sequential, two-alternative, forced-choice psychophysical procedure was used. The stimuli were brief (200-msec) two-frame sequences presented forwards (increasing smile) and backwards (decreasing smile) in pseudo-random order on each trial. Subjects were asked to report whether the first or second sequence of images was more pleasant. The strength of the preference for the forward order ranged from 50 to 100% for different sequences and depended on the between-frame variance. Rapidly increasing smiles (those with large variances) were more reliably perceived as pleasant than gradually increasing ones.

These results demonstrate that digital image analysis and psychophysics can be used to identify stimulus characteristics that define the boundaries between social messages in a rapidly changing signal. This method permits quantification of the hedonic qualities of facial expression.

TRANSPLANTATION: NEW TECHNIQUES, IMMUNE REJECTION AND BEHAVIOR

399.1

POLYMER ENCAPSULATED PC12 CELLS TRANSPLANTED IN MPTP LESIONED PRIMATES. P. Aebischer, M. Goddard, R. Timpson, A. Signore, A. Beauregard-Young, C. Rampone. Artificial Organ Laboratory, Brown University, Providence, RI.

Transplantation of polymer immunoisolated dopamine (DA) secreting cells may constitute an alternative for the treatment of Parkinson's disease. The encapsulation of a cell line within a permselective polymer membrane should not only restrict their outgrowth but prevent host rejection in cross-species transplantation. Chaired cynomologus monkeys were trained to pick food rewards from a multiwell plate. The time required to empty the wells was quantified several times per week. The neurotoxin MPTP was then injected via the right internal carotid artery which led to the development of a contralateral hemiparkinsonian syndrome. Six weeks following the MPTP injection, a semi-permeable membrane based U-shaped device was implanted stereotaxically in the DA-depleted striata. PC12 cells, a DA-releasing cell line derived from a rat pheochromocytoma, were then injected into the U-shaped device. A significant improvement in the ability of a severely lesioned primate to empty the wells was observed within the first week post-transplantation. Viable PC12 cells were recovered 3 months post-implantation by flushing the U-shaped device. This was followed by a deterioration of the monkey's performance at the picking test. Less severely lesioned monkeys showed mild improvement when either PC12 cells or dopamine releasing polymer rods were inserted in semi-permeable receptacles. These results show that polymer encapsulted PC12 cells can survive implantation into a primate striatum. Preliminary results indicate that the release of dopamine from a line source in the striatum is able to ameliorate the symptomatology of an MPTP induced Parkinson's syndrome in non-human primates.

399.2

NEURONAL SURVIVAL AND GROWTH AFTER DISSOCIATION AND REPLATING OF LONG TERM PRIMARY MESENCEPHALIC CELL CULTURES. C. Mytilineou, T. Bak, D. Casper and M. Blum. Dept. Neurology and Fishberg Center for Neurobiol., Mount Sinai Sch. Med. New York, N.Y., 10029.

The ability to maintain in storage a pool of a specific neuronal population would be important in brain transplantation. We have observed (Casper et al. Soc. Neurosci. Abst., 15:708, 1989) that exposure of rat mesencephalic cell cultures to epidermal growth factor (EGF) results in cell proliferation and confluence within 3 weeks in vitro. Staining with GFAP indicates that only a fraction of the cells are differentiated astrocytes. We considered the possibility that the apparently undifferentiated cells were both neuronal and glial precursors which could survive enzymatic removal and dissociation and differentiate after replating. Mesencephalic cells from E-16 rat embryos were grown in chemically defined medium with 10 ng/ml EGF. After 12, 14 or 17 days in vitro the cells were removed from the dishes by 0.06% trypsin, washed and resuspended in medium by trituration. $5X10^4$ cells were plated on polyornithine coated 35mm dishes in defined medium with10 ng/ml EGF. Both flat and phase bright cells attached to the dishes within 24 hrs and a large number of the phase bright cells began extending processes. 3-5 days after replating, cultures were stained with antibodies to GFAP or Tau to determine the presence of differentiated astrocytes and neurons. Most of the flat cells, about 20% of the total cell population, were positive for GFAP. A number of the phase bright cells were positive for Tau, had extended long processes and had the appearance of well differentiated neurons. Supported by NIH grants NS-23017; NS-07245 and United Parkinson Foundation.

399.3

GENE TRANSFER INTO BRAIN USING A HERPES SIMPLEX VIRUS (HSV) VECTOR. D.J.Fink, M.Mata, L.R.Sternberg*, W.Goins* and J.C.Glorioso*. VAMC, U.Michigan, Ann Arbor, MI 48104 and U.Pittsburgh, Pittsburgh, PA 15261.

We have exploited the natural biology of herpes simplex virus (HSV) to engineer recombinant HSV vectors of reduced neuropathogenicity for the transfer and expression of foreign genes in the mature CNS. The E. coli β-galactosidase (β-gal) gene (lacZ) driven by a viral late gene (gC) or the latency active (LAT) promoter was recombined into HSV as a reporter of foreign gene expression during productive and latent viral infection respectively.

In vitro, infected Vero cells showed intranuclear viral particles, and β-gal as demonstrated by the X-gal reaction product. ICP4 deficient pLAT:lacZ recombinants expressed β-gal in the absence of detectable viral particles.

In vivo, wild-type and glycoprotein C (gC) defective vectors caused focal brain destruction. Viral protein kinase (US3)-deficient pgC:lacZ recombinants expressed β-gal in neurons from 2 to 5 days after inoculation, but did not cause focal brain destruction, despite the fact that viral particles could be identified in cells expressing β-gal. pLAT:lacZ recombinants expressed β-gal at times consistent with viral latency.

These results show that HSV vectors can be used to express foreign genes in cells of brain.

399.4

EMBRYONAL CARCINOMA (EC) CELLS TRANSPLANTED INTO THE BRAIN DIFFERENTIATE INTO A NEURONAL LINEAGE: A POSSIBLE VEHICLE FOR EXOGENOUS GENES. B. Wojcik*, F. Nothias, J.F. Nicolas* and M. Peschanski, INSERM U161, 2 rue d'Alésia, 75014 Paris and Institut Pasteur, Paris France.

Cell lines potentially useful as vectors for the expression of exogenous genes after transplantation into specific brain regions should (i) readily incorporate and express foreign genes, (ii) lack tumorigenicity and (iii) be integrated into the host brain parenchyma. EC cells which differentiate into neural lineages, such as 1009, should meet these criteria.

To test this hypothesis, EC cells were infected with a replication defective retrovirus which expresses the nlsLacZ gene from the SV40 promotor. The β-gal gene product is localized to the nucleus. Cells expressing the exogenous gene could be selected via FACS using fluorescein di-galactosidase. In vitro, these cells displayed a stable expression of the nlsLacZ gene and retinoic acid-induced differentiation resulted in neuronal (neuron specific enolase NSE+) and glial (GFAP+) phenotypes. Implantation of β-gal+ EC cells was carried out in excitotoxin treated CNS areas of adult rats. The rats were perfused after one month and sections stained for β-gal followed by immuno-labeling of alternate sections with anti-NSE or anti-GFAP. β-gal+ EC cells were present in the transplants and some of them were double-labeled for NSE but not for GFAP. NSE labeling showed that EC cells which had differentiated into neurons extended neurites into the host parenchyma, a result confirmed by EM studies of HRP-prelabeled cells. These results show that transplanted 1009 cells lose their tumorigenicity and differentiate into a neuronal lineage after intracerebral grafting, while they are still able to express an exogenous gene.

399.5

IMMUNE PRIVILEGE IS EXTENDED TO INTRAOCULAR DEVELOPING RETINAL ALLOGRAFTS. L.Q. Jiang and J.W. Streilein, Dept of Microbiology & Immunology, Univ. of Miami School of Medicine, Miami, Fla 33101

One long term experiment goal of retinal transplantation is to be able to transplant viable neural retinal tissue into eyes blinded by retinal degenerative disorders. The survival of such grafts would be expected to depend partly upon the ability of the recipient's immune system to recognize the graft's alien transplantation antigens and to mount a destructive immune response. To examine the immune response evoked by intraocular retinal transplants, we transplanted histoincompatible neonatal BALB/c neural retina into the anterior chamber (AC) and subconjunctival (SC) space of adult C57BL/6 mice. Clinical and histologic examination indicated that even strongly histoincompatible developing neural retinal transplants, engrafted within the AC, acquired a blood supply, and differentiated into recognizable retinal structures , without the evidence of rejection. SC retinal grafts were rejected. To examine whether mice with intracameral retinal allografts developed a deviant immune response, a panels of C57BL/6 mice (5) received from histoincompatible developing neural retinal transplants into the AC, or in the SC. Twelve days later, the ears of these mice were tested for delayed hypersensitivity with intrapinnae injections of (C57BL/6xBALB/c)F1 spleen cells. A positive control panel of C57BL/6 mice that received BALB/c spleen cells SC or neonatal BALB/c retina implanted SC displayed vigour DH. By contrast, recipients of BALB/c retinal grafts into the AC failed to mount significant ear swelling responses. We next demonstrated that BALB/c graft-specific suppressor cells were generated in spleens of animals bearing AC retinal grafts, using an adoptive transfer assay. By contrast, recipients of normal spleen cells developed strong DH. We conclude that immune privilege is extended to the histoincompatible retinal grafts in AC, and that a deviant immune response, deficient in delayed hypersensitivity may play a protective role for intraocular retinal transplants.

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399.6

BROWN-NORWAY (BN) RATS DO NOT REJECT BRAIN GRAFTS FROM FISHER 344 (F344) RATS EVEN AFTER SYSTEMIC SENSITIZATION. M.Poltorak and W.J. Freed. NIMH Neuroscience Ctr. Washington, DC 20032.

Embryonic brain tissue allografts under many circumstances survive transplantation into the brain. It is generally believed that such grafts will not survive if the host animal is systemically sensitized, by skin grafting or other means, to major histocompatibility complex (MHC) antigens of the donor animal. We have found that F344 brain grafts survive in BN hosts even when the host is systemically sensitized to F344 tissue. Embryonic cerebral neocortex from F344 donors was transplanted into BN host rats (n=92). Subsequently, the host animals were systemically sensitized with donor skin (n=24), brain tissue (n=38), or spleen cells (n=6) and compared to a control group of allografts with no sensitization or sham procedures (n=24). Rejection of the transplants in BN rat hosts was not provoked by any of the sensitization methods tested. Minor immunological responses that did not result in rejection were, however, present in many host ani mals. We did not observe infiltration of W3/13+ T cells and 0X8+ cytotoxic lymphocytes in any of the groups. Nevertheless, substantial infiltrations of 0X6+ antigen-presenting cells and W3/25+ cells were present. There was also an extensive enhancement of MHC class I immunoreactivity in parts of the grafted tissue developing within the third ventricle, but not for the same type of graft in the lateral ventricle. These findings suggest that neural graft rejection depends on general genetic susceptibility to immune reactions, and not only to disparity between the antigens encoded by the MHC or minor MHC. Moreover, enhancement of MHC class I and class II expression within the transplanted tissue does not predict rejection of graft. (This study is dedicated to the memory of Cynthia R. Rodgers)

399.7

FIRST CLINICAL TRIAL OF MYOBLAST TRANSFER THERAPY: P.K. Law, Q. Fang*, T.G. Goodwin*, J.A. Florendo, H.Herrod*, T. Bertorini, D.S.Kirby*, H.J. Li*, M. Chen*, D. Yuan*, and G.S. Golden*. Depts. of Neurol. and Peds., Univ. of Tennessee, Memphis, TN 38163.

This double-blind study aims to determine the survival, development and functioning of donor myoblasts in dystrophic muscles of Duchenne Muscular Dystrophy (DMD) boys. We hypothesize that intramuscular injection of histoincompatible normal myoblasts can significantly improve the biochemistry, structure and function of dystrophic muscles. Our goal is to demonstrate the safety and efficacy of myoblast transfer in human.

Eleven DMD boys, aged 5 to 10, received myoblast injections in a randomly selected extensor digitorum brevis (EDB) muscle. The sham-injected EDB served as control. Donor myoblasts were derived from cell culture of muscle biopsies from the normal father/ward or normal brother of the host. Cyclosporine (CsA) treatment began two days before myoblast injection and continued for three months. Three days prior to the myoblast injection and three months afterward, the isometric twitch and maximum voluntary contraction of the left and the right EDBs were measured. Comparison of the mechanophysiology between the right and the left EDBs at 3 months after myoblast injection, and between the test EDB before and after myoblast injection would determine if the procedure exerted any beneficial effect on the dystrophic muscle. After mechanophysiologic testing at 3 months after injection, test and control EDBs were biopsied, and examined for dystrophin and for structural changes. If the procedure is proven successful, there will be potential for the use of myoblast transfer as a therapy to relieve dystrophic symptoms in other muscle groups in these patients. (Supported by MDA, Sandoz, and Walgreens).

399.8

SURVIVAL AND FUNCTION OF HUMAN MELANOCYTES IMPLANTED IN THE RAT STRIATUM. P.J. Kontur, C. Leranth, R. Halaban*, M.R. Lerner*, A.B. Lerner* and R.H. Roth. Depts. of Pharmacology, Dermatology, and Obstetrics and Gynecology, Yale Univ. Sch. Med., New Haven, CT 06510.

Melanocytes may serve as an alternative to adrenal or neuronal cells for transplantation in animal models of Parkinson's disease because they have the capacity to synthesize L-DOPA through the actions of the enzyme tyrosinase. The ability of normal human melanocytes to survive and produce L-DOPA after striatal implantation was examined in non-immunosuppressed rats with and without unilateral 6-hydroxy-dopamine-induced lesions of nigrostriatal dopamine neurons. The ability of melanocytes to affect apomorphine-induced rotation after implantation into the striata of lesioned rats was also evaluated. Melanocytes implanted in normal and lesioned striata survived but did not appear to proliferate when examined at various times post-implantation. Melanosomes, the site of tyrosinase activity, were present in melanocytes implanted in both normal and lesioned striata. However, melanosomes in the striata of non-lesioned rats appeared to be degenerating and lymphocyte infiltration was present. Rats with melanocyte implants in lesioned striata showed reductions in the contralateral rotation induced by the injection of apomorphine. These data suggest that melanocytes may serve as an alternative transplantable cell source of DOPA for the treatment of Parkinson's disease. Supported in part by USPHS Grant MH 14092, NS26068 and the United Parkinson Foundation.

399.9

INTRAHIPPOCAMPAL GRAFTS OF SEPTAL TISSUE TAKEN AT DIFFERENT EMBRYONIC AGES: EFFECTS ON BEHAVIORAL DEFICITS INDUCED BY ELECTROLYTIC FIMBRIA-FORNIX LESIONS IN THE RAT. J.C. CASSEL*, C. KELCHE*, I. GOEPP*, B. WILL. D.N.B.C., Centre de Neurochimie du C.N.R.S., 12, rue Goethe, F-67000 STRASBOURG (FRANCE).

Starting three months after grafting, behavioral effects *(home cage and open field activity, radial maze learning)* of intrahippocampal grafts of fetal ED14 *(Group S14)* and ED16 *(Group S16)* septal-diagonal band tissue, or of cortical tissue, as a control graft *(Group Cx)*, were assessed in Long Evans female rats given electrolytic lesions of the dorsal subcallosal septo-hippocampal pathways *(Group L)*. Sham-operated non-grafted rats served as controls *(Group S)*.

Compared to S rats, L rats showed increased activity in both their home cage and the open field, as well as dramatic impairment in maze learning. Neither type of graft provided any benefit on home cage activity, but graft-induced improvement was observed in the open field scores only in S16 rats and on maze learning scores only in S14 rats. These results, which will be discussed in the light of histological data, suggest that beneficial effects of septal grafts on fimbria-fornix lesion-induced behavioral deficits may be expressed differentially as a function of the fetal donor's maturity stage.

399.10

HOMOTOPIC NEURAL GRAFTS DO NOT CONSISTENTLY IMPROVE FORELIMB REACHING PERFORMANCE OF RATS WITH EXCITOTOXIC LESIONS OF THE STRIATUM. M. Pisa, P. McLean and J. A. Schranz. Dept. Biomedical Sciences, McMaster University, Hamilton, Ont., Canada, L8N 3Z5.

Female Wistar rats with bilateral, quinolinic acid-induced, excitotoxic lesions of the rostrolateral striatum were assigned to groups at random for homotopic injections of E14 cell suspensions of either striatal primordium (homotypic grafts) or cortex (heterotypic grafts) or cell-suspension vehicle (lesion-only group). The heterotypic grafts were unilateral and the homotypic grafts either unilateral or bilateral. The reaching performance of these rats was compared with that of control rats with neither lesions nor grafts, about 1 year after surgery. The lesions produced a significant impairment. The rats with either bilateral homotypic grafts or unilateral heterotypic grafts performed more poorly than the controls and did not reliably differ from the rats with lesions only. In contrast, the rats with unilateral homotypic grafts did not significantly differ from the controls. However, these rats preferentially performed with the paw contralateral to the nongrafted striatum. Histology confirmed both the lesions and the survival and growth of all grafts. Homotopic neural grafts do not appear to benefit the reaching performance of rats with striatal lesions, at least not consistently. (Supported by the MRC of Canada. M. Pisa is a Research Associate of the Ontario Mental Health Foundation).

399.11

IMPLANTED INTRASTRIATAL MICROENCAPSULATED DOPAMINE REDUCES DA AGONIST- INDUCED ROTATIONAL BEHAVIOR A. McRae[1], S. Hjorth[2], D. Mason*[3], L. Dillon*[3], T. Tice*[3] University of Göteborg, Dept of Histology[1] and Pharmacology[2] Göteborg, Sweden 40033 and Southern Research Institute[3] Birmingham Alabama USA 35255.

Dopamine (DA) has been encapsulated in 2 different copolymer [poly (DL lactide-co-glycolide)] excipients; one with a preprogrammed biodegradation time of 6 weeks (DA 50:50), and the other one expected to last about 3 months (DA 65:35). We compared the correspondence between the predicted time of co-polymer biodegradation and the duration of time that intrastriatally implanted DA microcapsules were able to reduce DA agonist-induced rotational behavior in unilaterally 6-hydroxydopamine (6-OHDA) lesioned rats. Rats displaying ≥ 400 contralateral rotations to apomorphine challenge were administered a suspension of DA microspheres in the mediocentral striatum. The rats were challenged with apomorphine on a weekly basis. The group receiving DA 50:50 returned to baseline levels 4 weeks after implantation. The group receiving DA 65:35 displayed significant reductions in contralateral rotional behavior beyond 8 weeks after implantation. Rats receiving empty microspheres did not demonstrate any significant change in the number of contralateral rotations. Similar results were obtained with the DA D-2 agonist 3-(3-hydroxyphenyl)-N-n-propylpiperidine (-) 3-PPP). We found no attenuation in the rotational responses to apomorphine 3-40 days after lateral striatal DA 50:50 microcapsule implants. These results support the concept of functional heterogeneity of the striatum. All of these results indicate that DA microsphere preparations have the potential of being employed as a source of neurotransmitter replacement with the CNS. These formulations allow sustained diffusion of microencapsulated DA into the CNS at a controlled rate for pre-determined periods of time and assure functional significance.

399.12

TRANSPLANTED RETINAE: ASSESSMENT OF FUNCTIONAL INTERACTIONS WITH HOST OPTIC INPUT AND SENSITIVITY TO ILLUMINATION. J.D. Radel, R.D. Lund and S. Das*. Dept. Neurobiology, Anatomy & Cell Science, Univ. Pittsburgh Sch. Med., Pittsburgh, PA 15261.

We report results from studies in which pupillary responses of adult host rats, elicited by illumination of intracranial retinal transplants, were recorded over a several week period in the same animal. If one host eye is removed at the time of transplantation (on postnatal day 1) an extensive transplant innervation of several host visual nuclei occurs, and a robust transplant-mediated pupilloconstriction is often present in the mature host as a result. The pupillary reflex typically shows little change after all host optic input is subsequently abolished by intracranial optic nerve section, and remains stable for at least a 5 week period. Retinae transplanted into newborn hosts having both eyes intact innervate the host brain poorly and mediate only minimal changes in pupillary diameter when illuminated after the host has matured. A marked improvement in the response can, however, be observed within 24 hrs after the optic nerves are sectioned. The time course of this change is too rapid to be attributable to anatomical sprouting of the transplant projection. We are presently studying these responses at longer periods after optic nerve section to determine if sprouting of the transplant projection induced by removal of the host inputs results in further changes in the transplant-mediated response. (Supported by NIH grants EY05283, EY05967 & HD07343)

399.13

TRANSPLANTATION OF FETAL XENOGRAFTS FROM THE VISUAL CORTEX OF RATS TO THE VISUAL CORTEX OF ADULT CATS: RESPONSIVENESS OF CORTICAL CELLS IN THE HOST BRAIN. U. YINON and R. SHEMESH*, Physiol. Lab., Goldschleger Eye. Res. Inst., Tel-Aviv Univ. Fac. Med., Sheba Med. CTR, Tel-Hashomer, 52621, Israel.

Fetal (E15-E17) rats' visual cortex grafts were injected into a section unilaterally made in visual cortex area 17 of 4 adult cats. As sham controls 4 adult cats in which an equivalent section was made were also studied. Electrophysiological unit recordings were extracellularly performed, 4-9 weeks after surgery. Typical patterns of action potential discharges were identified in the cortex of all cats studied. While medial to the transplant bridge (in the visually deafferented area) the majority of the recorded cells were spontaneously active (only 29.5% were visually responsive), a reasonable activity (64.8% responsive cells) was found lateral to it and an almost normal activity (75% responsive cells) in the untouched hemisphere. The performance of the cells was much higher in the analogous regions of the sham cats; 57.3% of the cells medial to the section, 86% lateral to it and 79.3% of them in the untouched hemisphere were visually responsive. In conclusion, the physiological results, although reflecting immunological reaction of the host cells, indicated no critical effect attributed to the presence of the xenograft in the host cortex.

400.1

FOLATE STIMULATION OF GLUCOSE UTILIZATION AND TRANSPORT IN SYNAPTOSOMES, CULTURED C6 CELLS AND ASTROCYTES. S.R. Snodgrass and M.H. Morita*, Neurology Research Laboratory, Childrens Hospital Los Angeles and Depts. of Neurology and Pediatrics, USC School of Medicine, Los Angeles, CA

Folates are known to produce CNS excitation, convulsion and brain lesions. Nonmethylated folates such as folic acid (FA) are more potent neurotoxins than physiological folates. We have found folate stimulation of ^{45}Ca uptake, cGMP accumulation, and phosphate transport. We studied folate effects on 2-deoxyglucose accumulation (DGA) in rat C6 cells, astrocytes, and synaptosomes. FA and methotrexate (MTX) produced 32% and 40% increases at 10 uM concentration (10 min preincubation, 45 min incubation, effect lost if preincubation exceeded 30 min). Higher FA and MTX concentrations produced larger increases, up to 3 fold. The physiological folate 5-methyltetrahydrofolate (MTHF) was a weaker stimulator of DGA. Pteridine, a part of the folate molecule, also caused dose dependent increases of DGA. Folate responses were smaller in astrocytes and synaptosomes than C6 cells but statistically significant. Folates produced more stimulation of U-^{14}C-glucose accumulation than of DGA, and greater effects on DGA than on 3-O-methyl glucose accumulation. Folates stimulate both glycolysis and glucose transport in several neural tissues. The effect is insensitive to ouabain and dihydropyridines but inhibited by chlorpromazine and other neuroleptics.

400.2

ACTIVATION OF ARACHIDONIC ACID METABOLISM BY PAF AND CALCIUM-IONOPHORE IN PRIMARY CULTURE OF ASTROGLIAL CELLS. A. Petroni*, M.Blasevich*, F.Visioli,* B.Zancocchia,* G.Racagni and C.Galli *. Institute of Pharmacological Sciences, University of Milan, 20133, Milan, Italy.

Astrocytes actively metabolize arachidonic acid (AA) via the lipo (LO) and cyclooxygenase pathways after appropriate stimulation.CO and LO metabolites play important roles in physiopatological conditions such as cerebral ischemia, convulsions, acute inflammation etc. Moreover LO products have been proposed as intracellular second messengers in Aplysia (Piomelli et al. Nature 328:38, 1987) and as intercellular modulators (Maclouf et al. Pharm. Res. 21:1,1989).

We have measured the formation of AA metabolites following activation of phospholipases (PLases) and conversion through the LO and CO pathways, in primary cultures of astroglial cells, stimulated with the Ca-ionophore A-23187 and PAF (platelet activating factor).Cells were obtained from brain cortex of one day old rats and were characterized by immunofluorescent staining. The activation of PLA_2 and C,by A 23187 and PAF, was investigated incubating the cells with tritiated AA and evaluating the radioactivity in labelled metabolites. Incubation with PAF and A 23187 were carried out at different concentrations and for different time periods. The LO products hydroxy-eicosatetraenoic acids were measured by HPLC and GC-MS , LTC_4 by RIA. Levels of the CO metabolites, 6-keto-$PGF_{1\alpha}$, TXB_2 and PGD_2 were measured by RIA or EIA. PAF and A 23187 differentially activated the release of AA from phospholipids and the formation of diacylglycerol, suggesting stimulation of PLA_2 and PLC. The activation of CO and LO pathways was dependent from the time and the concentration of the stimuli . In addition , AA metabolism was affected by preincubating the astroglial cells with PAF antagonists and ganglioside derivatives.

This work was supported by FIDIA S.p.A., Abano Terme, Italy.

400.3

SOMATOSTATIN (SS) AUGMENTATION OF THE M-CURRENT (I_M) IN HIPPOCAMPUS: MEDIATION BY A LEUKOTRIENE. P. Schweitzer*, S. Madamba* and G.R. Siggins. Dept. of Neuropharmacology, Scripps Clinic Research Institute, La Jolla, CA 92037.

We previously reported that SS causes an outward current (hyperpolarization) with an increase in conductance and I_M amplitude in CA1 pyramidal neurons (PNs) in a slice preparation (Moore et al., Science 239:278, 1988). We used single electrode voltage clamp to determine if the SS effects involved a second messenger. Superfusion of arachidonic acid (AA), a product of phospholipase, augmented I_M and caused an outward baseline current with a conductance increase. These effects were not altered by CsCl 2mM. Quinacrine and 4-bromophenacyl bromide, inhibitors of phospholipase A_2, both blocked the I_M action of SS and the outward current. AA is metabolized to prostanoids via cyclooxygenase, and to hydroxy- and hydroperoxy-eicosatetraenoic acids (HETE, HPETE) and leukotrienes via lipoxygenase enzymes. The I_M effects of AA and SS were blocked by nordihydroguairetic acid, a non-specific lipoxygenase inhibitor, but not by the cyclooxygenase inhibitor indomethacin. Several prostaglandins (PGE_2, $PGF_{2\alpha}$, PGI_2) had no effect on I_M. These data suggest that a lipoxygenase product mediates the M-current augmenting effect of SS. In support of this, leukotriene C_4, but not B_4, markedly augmented I_M. 12 HPETE hyperpolarized PNs but did not alter I_M. Two specific inhibitors of 5 lipoxygenase (the enzyme producing leukotrienes), 5,6-methanoleukotriene A4 methylester and 5,6-dehydroarachidonic acid, blocked the I_M effect of SS and AA. These data implicate an AA metabolite, probably a leukotriene, in the SS-induced increase in I_M in hippocampal PNs. Supported by NIMH (MH44346), NIAAA (AA06420) and N.A.T.O.

400.4

DOES cGMP CONTRIBUTE TO THE DESENSITIZATION OF BRADYKININ ELECTROPHYSIOLOGICAL RESPONSES IN DRG NEURONS? D.S. McGehee, M.F. Goy, and G.S. Oxford. Dept. of Physiology, University of North Carolina, Chapel Hill, NC 27599

The nonapeptide bradykinin (BK) has been shown to cause two types of electrophysiological responses in a subpopulation of cultured rat DRG neurons: 1) stimulation of an inward cation current, and 2) inhibition of voltage-activated calcium currents. Both of these responses desensitize in 1-3 minutes, despite the continued presence of agonist. Furthermore, responses to second applications are attenuated or suppressed for up to 10 min. after washout of the first application. In parallel with these electrophysiological responses, BK has been shown to stimulate a rapid release of phophotidylinositol metabolites from these cultures with peaks of IP3 and diacylglycerol at 5 and 20 seconds, respectively (Gammon et al., *J. Neurochem.* **53**:95). The biochemical response also desensitizes, and it has been proposed that cGMP negatively feeds back on this pathway (Burgess et al., *J. Neurosci.* 9:3314).

We are interested in what role cGMP might play in electrophysiological desensitization. We have measured cGMP production from DRG neurons stimulated with BK and see increases of approximately five-fold which peak at 30 seconds and return to baseline by 1 minute. These responses also show strong desensitization. Nitroprusside, which mimicks the BK-induced cGMP increase, also mimicks the BK-induced desensitization of the cGMP signal. Pretreatment of the cultures with 100μM methylene blue for 1 min. blocks the BK-induced desensitization. Utilizing these and other compounds to manipulate cGMP synthesis, we have analyzed the role of cGMP in the desensitization of BK-induced biochemical and electrical responses. Supported by NIH (NS23804 and NS21290) and GLAXO, Inc.

400.5

MUSCARINIC-STIMULATED CALCIUM MOBILIZATION IS NOT COUPLED TO CYCLIC GMP GENERATION IN N1E-115 CELLS. A.D. Morielli and S.H. Thompson. Hopkins Marine Station, Stanford University, Pacific Grove, CA 93950

Calcium dependence of cGMP generation in N1E-115 cells is well documented, but carbachol-induced changes in cytoplasmic Ca levels have not been previously observed. We find that Ca is mobilized from internal stores by pirenzepine-sensitive muscarinic receptors. Using the calcium indicating dye fura-2, we measured a mean resting Ca of 56nM. 70% of the carbachol-treated cells mobilize calcium to peaks of greater than 600 nM within 5 seconds. Pronounced calcium oscillations occur in about 20% of the cells. In many cases, a wave of Ca release followed by Ca uptake produces a band of Ca which traverses the cell multiple times. Removal of external Ca has no effect on this response.

Surprisingly, we found that cGMP generation by carbachol is absolutely dependent on external calcium, despite the mobilization of calcium from internal stores. Using electrophysiological techniques, we found that carbachol also causes calcium influx. Bromophenacyl bromide and melittin, drugs that influence phospholipase A2 activity, also cause a profound and tonic elevation in resting calcium levels via calcium influx, and therefore have limited utility as probes of PLA2 involvement in cGMP generation. We conclude that cGMP generation depends on Ca influx, and not on Ca mobilization from internal stores, although both events occur in response to receptor activation. It is possible that the same Ca-ATPase activity which produces traveling waves of Ca acts as a barrier to the diffusion of Ca released from internal stores to the inner surface of the plasma membrane.

400.6

REGULATION OF THE MUSCARINIC-INDUCED CALCIUM "FINGERPRINT" IN N1E-115 MOUSE NEUROBLASTOMA CELLS. S.S.-H. Wang, A. Rocco Morielli, and S.H. Thompson. Hopkins Marine Station, Stanford University, Pacific Grove, CA 93950.

We are investigating the mechanisms of calcium mobilization through the phosphatidyl inositol (PI) cascade. Fluorescence imaging, microperfusion, and flash photolysis are used to observe the response of single N1E-115 cells to the muscarinic agonist carbachol (CBC). Cells are loaded with the calcium indicator fura-2 or fluo-3. 1 mM CBC is applied either directly to the bath or with a rapid, focal perfusion micropipette. The spatiotemporally complex "fingerprints" observed are reproducible in separate drug applications 30-60' apart (bath) or 30-60" apart (microperfusion). With microperfusion, Ca transients far outlast the presence of agonist in the bath. Return to basal $[Ca^{++}]_i$ after a spritz of CBC is followed by a refractory period during which the time course of Ca mobilization is altered.

We are using flash uncaging and microinjection of Ca and phosphoinositides to assess the role of second-messenger cascades in Ca mobilization, by inducing step changes in PI metabolites and Ca.

400.7

N-METHYL-D-ASPARTATE RECEPTORS CAUSE A TRANSIENT ACTIVATION OF NITRIC OXIDE SYNTHETASE AND CYCLIC GMP ACCUMULATION IN CEREBELLAR GRANULE CELLS. L. Kiedrowski, E. Costa and J. T. Wroblewski. Fidia-Georgetown Institute for the Neurosciences, Georgetown Univ. Sch. of Med., Washington, DC 20007.

The stimulation of *N*-methyl-D-aspartate (NMDA) receptors in cerebellar granule cells activates guanylate cyclase and increases cyclic GMP content. To establish whether this signal transduction is mediated through the activation of nitric oxide (NO) synthetase which generates NO, an activator of soluble guanylate cyclase, the enzyme activity was measured in intact primary cultures of cerebellar granule cells equilibrated with [^{3}H]arginine by following its conversion to [^{3}H]citrulline. Activation of NMDA receptors by glutamate or NMDA increased in a dose-dependent manner the formation of [^{3}H]citrulline and the cyclic GMP accumulation, measured in the same samples. Both effects were blocked by the NMDA receptor antagonist MK-801 (1 μM) and the NO synthetase inhibitor N^G-methyl-L-arginine (3 μM). The activation of NO synthetase was also evoked by the calcium ionophore A23187. The maximal conversion of the radioactive label into [^{3}H]citrulline occurred 5 min after the application of the agonist and then remained constant for 60 min. Further enzyme activation was not produced by subsequent additions of glutamate or the calcium ionophore. These results indicate that the activation of NMDA receptors leads to a transient increase in the activity of NO synthetase. Since cyclic GMP accumulation showed a similar transient responsiveness to glutamate, one might infer that NO synthetase desensitizes.

400.8

DUAL REGULATION OF GUANYLATE CYCLASE BY *N*-METHYL-D-ASPARTATE RECEPTORS: ROLE OF NITRIC OXIDE AND ARACHIDONIC ACID. B. Wroblewska, M. Majewska*, E. Costa and J. T. Wroblewski. Dept. of Physiology and Fidia-Georgetown Institute for the Neurosciences, Georgetown Univ. Sch. of Med., Washington, DC 20007.

In primary cultures of cerebellar neurons the stimulation of *N*-methyl-D-aspartate (NMDA) receptors leads to the activation of guanylate cyclase and cyclic GMP accumulation. This stimulation was reduced in a dose-dependent manner by N^G-methyl-L-arginine, an inhibitor of nitric oxide (NO) synthetase, indicating that cyclic GMP accumulation depends on NMDA-stimulated NO formation. Accordingly, cyclic GMP accumulation was induced by sodium nitroprusside (SNP), which acts by releasing NO that directly activates guanylate cyclase. The stimulation of cyclic GMP formation by NMDA was also reduced by nordihydroguaiaretic acid, the inhibitor of lipoxygenases, indicating that the mediation of the receptor signal requires also an active metabolism of arachidonic acid. The maximal cyclic GMP accumulation induced by SNP was 3 to 4 fold higher than that elicited by NMDA, however, when both compounds were added together the effect was not additive. In fact, NMDA caused a dose-dependent inhibition of SNP-activated cyclic GMP formation, reaching at maximal concentrations the level stimulated by NMDA alone. Similar results were obtained in presence of 3-isobutyl-1-methyl-xantine, a phosphodiesterase inhibitor, suggesting that the interaction affects cyclic GMP formation and not its degradation. These results suggest that the NMDA receptor-induced regulation of guanylate cyclase activity may involve the interaction of two messengers, NO and a lipoxygenase product of arachidonic acid metabolism.

400.9

GLUTAMATE MODULATES ENDOGENOUS ADP-RIBOSYLATION IN CEREBELLAR NEURONS. R. Raulli, M. Majewska*, W. J. Wojcik, E. Costa and J. T. Wroblewski. Fidia-Georgetown Institute for the Neurosciences, Georgetown Univ. Sch. of Med., Washington, DC 20007.

The activation of glutamate receptors in cerebellar granule cells stimulates several signal transduction systems including the generation of nitric oxide (NO). Moreover, agents such as sodium nitroprusside (SNP), which release NO, were shown to activate an endogenous cytosolic ADP-ribosyltransferase in a variety of tissues. In order to determine whether glutamate affects endogenous ADP-ribosylation, granule cells were incubated for 20 min in the absence or presence of glutamate. They were then harvested, homogenized, and centrifuged, and the ADP-ribosylation was monitored *in vitro* in the presence of [^{32}P]NAD in cytosol fractions incubated for 30 min at 37°C with SNP, and was analyzed by SDS-PAGE. SNP caused the ADP-ribosylation of several proteins present in the cell cytosol, the most prominent being a 40 kDa band. This effect was not mimicked by cyclic GMP, indicating that SNP-induced ADP-ribosylation is not mediated through the activation of guanylate cyclase, but it may be triggered by NO action. In granule cells treated with glutamate the ADP-ribosylation induced *in vitro* by SNP was decreased, suggesting the ability of glutamate to ADP-ribosylate the protein in intact cells, possibly through generation of NO. Similar experiments performed with cholera toxin which ADP-ribosylates specific GTP-binding proteins, showed two radioactive bands (45 and 61 kDa) present in the cell homogenates. Glutamate treatment preferentially decreased the appearance of the 61 kDa band. These results suggest that stimulation of glutamate receptors may increase either the activity of intracellular ADP-ribosyltransferases or the expression of their protein substrates, including the regulatory GTP-binding proteins.

400.10

NEURAL LOCALIZATIONS OF NITRIC OXIDE SYNTHASE SUGGEST GENERAL ROLE AS INTERCELLULAR MESSENGER. D. S. Bredt, P. M. Hwang, J. Buzy, & S. H. Snyder, Johns Hopkins Sch. of Med.

Nitric oxide (NO) is a major, if not the sole source of endothelium derived relaxing factor (EDRF) which mediates the vasodilating effects of neurotransmitters on certain blood vessels. Cyclic GMP elevations in the cerebellum and in neuroblastoma cell lines in response to calcium mobilizing neurotransmitters is also mediated by NO. Recently, we purified NO synthase (NOS; EC#1.14.23) to homogeneity from rat brain (Bredt and Snyder *PNAS* 87:682-685, 1990). Our ability to purify NOS was due to the development of a simple, sensitive, and specific assay monioring conversion of [3H]arginine to [3H]citrulline, which occurs stoichiometrically with the formation of NO, and our discovery that NOS is a calmodulin requiring enzyme. We have raised and affinity purified two antisera which immunoprecipitate NOS activity and recognize a single 150 kD band in western blots of numerous brain regions and peripheral tissues.

Immunohistochemistry in the brain shows NOS is discretely localized and is absolutely restricted to neurons and vascular endothelial cells. Highest density occurs in the cerebellum, olfactory bulb, and superior and inferior colliculi. In the periphery, NOS is highly enriched in the posterior pituitary, in the choroid of the eye, in the myenteric plexus and associated neuronal processes in the intestine, and in vascular endothelial cells. Microscopic localization shows the enzyme is most highly enriched in neuronal axons. Dense staining occurs in glomerular synapses in the cerebellum, nerve terminals in the granule cell layer of the olfactory bulb, nerve processes in the posterior pituitary, and in neuronal axons surrounding large cerebral blood vessels. Calcium mobilization in these nerve terminals may generate NO as a unique neurotransmitter.

400.11

CHARACTERIZATION OF THE INHIBITION OF THE EAA RECEPTOR LINKED TO PI METABOLISM BY 2-AMINO-3-PHOSPHONOPROPIONATE IN THE RAT HIPPOCAMPUS. E. Palmer* and C.W. Cotman, Department of Psychobiology, University of California, Irvine, CA 92717.

Excitatory amino acids (EAA) interact with specific membrane receptors to either operate ion channels directly linked to EAA receptors (ionotropic receptors) or to generate second messengers via phosphoinositide (PI) metabolism.The most potent agonists for the PI-linked receptor are *trans*-1-aminocyclopentyl-1,3-dicarboxcylic acid (ACPD), ibotenic acid (IBO) and quisqualate (QA). Of these, the most selective agonist for PI stimulation is ACPD. It has been reported that 1 mM L-2-amino-3-phophonopropionate (AP3) is a very efficient inhibitor of IBO and QA stimulation of PI metabolism and that 2- amino-4-phosphonobutyric acid (L-AP4) also inhibits PI metabolism, but not as effectively.

Using hippocampal slices from 8-10 day old rats we confirm that AP3 inhibits PI stimulation by IBO and QA, although lower levels of inhibition were observed than those previously reported. We further report that AP3 also inhibits the stimulation of PI metabolism by ACPD. Our data indicate that the mechanism of inhibition by AP3 is not via the AP4 receptor as inhibition of IBO, QA and ACPD induced stimulation by L-AP4 was not observed. Incubations with 1 mM AP3 performed in the presence of excess agonist did not result in the reversal of the inhibition. Inhibition of ACPD and QA stimulation by AP3 was temperature dependent, while that of IBO was not. Thus, it appears that AP3 inhibits via a complex mechanism that is neither competitive nor reversible.

400.12

INHIBITION OF GLUTAMATE RELEASE FROM HIPPOCAMPAL MOSSY FIBER SYNAPTOSOMES BY 12-HETE. E. J. Freeman, D. S. Damron*, D. M. Terrian and R. V. Dorman. Dept. Biological Sciences, Kent State Univ., Kent , OH 44242.

Superfused rat hippocampal mossy fiber synaptosomes were used to investigate the role of lipoxygenase products in the evoked release of endogenous glutamate. Pretreatment of the synaptosomes with 12(R)- or 12(S)-HETE (10 uM) reduced the 35 mM K^+-evoked glutamate release by 29% and 20%, respectively. These effects appeared to be specific for 12-HETE, since neither 5- nor 15-HETE were able to affect glutamate release. In addition, inhibition of lipoxygenase activity with 10 uM NDGA potentiated the 35 mM K^+-evoked glutamate release by 83% and the 200 uM arachidonate-induced release by 113%. The observation that 12-HETE inhibited the 35 mM K^+-induced Ca^{2+} influx by 79% supports the suggestion that it modulates membrane potential and inhibits neurotransmitter release. Also, blocking K^+ channels with 4-aminopyridine or 3,4-diaminopyridine stimulated the release of glutamate. Therefore, it appears that 12-HETE acts as an inhibitory signal to decrease glutamate release from hippocampal mossy fiber terminals. The above results are consistent with the hypothesis that lipoxygenase products activate presynaptic K^+ channels, which mediates an inhibition of neurotransmitter release. Supported by AFOSR 89-0245.

401.1

SPATIAL RESPONSE PROPERTIES AND PROJECTION PATTERNS OF SECONDARY MEDIAL VESTIBULOSPINAL TRACT (MVST) NEURONS. S.I. Perlmutter, Y. Iwamoto*, J.F. Baker, B.W. Peterson, Northwestern Univ. Med. School, Chicago IL

To determine how spatial motor patterns of the vestibulocollic reflex are produced, we studied 59 MVST neurons activated monosynaptically from the labyrinth in decerebrate cats. Projections were identified by antidromic activation from, or by recording intraaxonally in, ipsi- or contralateral C1 MVST, and by responses to electrical stimulation of IIIrd nucleus and C6 segment. A vector representing the rotation producing a neuron's maximal activation was derived from its response to 0.5 Hz rotations in many vertical and horizontal planes. In some cats horseradish peroxidase (HRP) was then injected intraaxonally at C1.

Neurons with different projections responded differently. Of 28 neurons projecting to IIIrd nucleus, 27 were not activated from C6 and had a response vector similar to that of an ipsilateral canal. Vectors of neurons projecting neither to IIIrd nucleus nor C6 (n=19) were similar, but more often indicated considerable vertical-horizontal canal convergence. 12 of 13 neurons which did project to C6 were excited strongly by yaw rotation to the contralateral side (type II response), or had convergent input from 2 vertical canals. Four secondary MVST axons were stained with HRP. Each sent branches to a specific region of the C1 ventral horn. For example, one axon with primarily posterior canal input had dense arborization and numerous terminals in the dorsomedial ventral horn, where motoneurons of longus capitis, a head flexor, are located.

Data suggest that secondary vestibulocollic neurons primarily relay activity of a single ipsilateral semicircular canal, as do vestibuloocular neurons reported previously. Signals with more canal convergence are carried past neck segments. NS17489, EY06485, EY05289, EY07342

401.2

OTOLITH CONTROL OF HEAD-NECK POSTURE IN THE GUINEA PIG. D.H. Wang, W. Graf, C. de Waele*, P.P. Vidal and L.C. Evinger. The Rockefeller University, New York, NY 10021; Lab. Physiologie Neurosensorielle, CNRS, 75270 Paris, France; SUNY, Stony Brook, NY 11794.

Previous cineradiographic studies indicated that at least five functional degrees of freedom are necessary to describe head/neck movements in most mammals. Thus, the vestibular origins for each postural control parameter needed to be determined. Selective ablations of utricular and saccular otoliths were performed by centrifuguing anesthetized animals. Succesful ablations were confirmed by testing postural reflexes and later on by histology.

Subsequent cineradiographic imaging of the lesioned animals indicated that the utricular otolith contributes mainly to controlling both head and neck posture in the sagittal plane, while the saccular otolith controls head orientation in the frontal plane. Thus, bilateral utricular otolith damage eliminated the vertical orientation of the cervical column in the sagittal plane. In addition, a vertical head nystagmus was observed indicating a lifting effort when the head was gradually pulled down by gravity. Unilateral sacculus ablation resulted in a non-horizontal head posture in the frontal plane. Our results suggest that the vertical orientation of the neck and the horizontal orientation of the head are controlled independently from each other as well as from dynamic variables (such as movement speed, etc) by a modular control strategy. - Supported by USPHS grant EY-04613.

401.3

NEURONAL DEATH DURING THE DEVELOPMENT OF THE VESTIBULAR EFFERENT SYSTEM OF THE RABBIT. N.H. Barmack, RS Dow Neurological Sciences Institute, Good Samaritan Hosp.& Med. Ctr., Portland, OR 97209.

The location and the approximate numbers of the neurons which comprise the vestibular efferent system (VES) have been described for several mammals. The VES consists of approximately 100-400 neurons which can be identified by retrograde transport of HRP injected into the labyrinth. Vestibular efferent neurons (VENs) are cholinergic, have somatic diameters of 6-9 μm and are found lateral to the abducens nucleus, ventral to the medial vestibular nucleus and border the medial aspect of the facial nerve.

We have used an antibody to choline acetyltransferase (ChAT) and retrogradely transported HRP as markers for VENs and we have studied these neurons during postnatal development of the VES in rabbits (6-35 days). In neonatal rabbits VENs comprise a well defined nucleus. There was good quantitative agreement (less than 10% difference) in counting cells filled with HRP and cells stained for ChAT. We have counted as many as 600 VENs in 8-14 day old rabbits (N=5). However, in 21-35 day old rabbits only 50-200 VENs could be identified with the same methods (N=6). Previous experiments have noted that the vestibular efferent projection is bilateral with approximately 50% of the projection terminating in each labyrinth. However, in individual rabbits the ratio of contralaterally to ipsilaterally projecting VENs can vary by a factor of four. The decrease in the number of VENs during development and the variability in the ipsilateral/contralateral projection pattern may reflect important cellular events linked to the innervation of the peripheral vestibular apparatus. However, the death of VENs may also reflect one of the cellular mechanisms by which the vestibular primary afferent system is "calibrated" during development, and therefore might be influenced by neuronal activity in the peripheral or central vestibular system.

401.4

THE RESPONSE OF VESTIBULAR NUCLEUS NEURONES TO SENSORY CONFLICT. L.R. Harris and J.W. Stelling* Department of Physiology, University of Wales, Cardiff, UK

The vestibular nucleus (VN) is a site of convergence of canal, otolith and visual signals. The time constant of the VN's response suggests velocity storage. We recorded from 66 type I VN cells in 7 cats during sensory conflict. Canal/visual conflict was obtained by rotating at constant velocity in the light until the canals' response had declined to zero and then suddenly slowing. Canal/otolith conflict was obtained by rotation about an off-vertical axis before suddenly slowing. At the point of slowing, the canals indicated rotation commencing in the opposite direction whereas vision or otolith signals were veridical. By adjusting the initial and final velocities, a greater or lesser degree of conflict could be achieved. Eye movements showed attenuation during large conflicts which was not seen in the VN cells' responses, suggesting that the site of effect of the conflict detection is beyond the vestibular nucleus.

401.5

SODIUM, CALCIUM, AND POTASSIUM ION INFLUENCES UPON TRANSMISSION AT THE HAIR CELL-AFFERENT FIBER SYNAPSE OF THE FROG. S.L. Cochran. Dept. Life Sciences, Indiana State Univ., Terre Haute, IN 47809.

This study is directed towards understanding how ions are involved in transmission between hair cells and afferent fibers in the isolated vestibular labyrinth of the frog, *Rana pipiens*. Intracellular recordings from lagena and canal afferents are digitized at 50 KHz for several minutes. EPSPs (several thousand per cell) are detected by computer algorithm and their frequency of occurrence and amplitudes are quantified. Increasing the Na^+ concentration of the solution bathing the tissue by 10-50 mM results in an increase in the mean amplitude of the EPSPs (19 cells), suggesting that the hair cell transmitter opens subsynaptic Na^+ channels. Reducing the Ca^{+2} concentration from 2 to 0.125 mM results in an increase in EPSP amplitude (22 cells), while increasing the Ca^{+2} concentration to 4 or 8 mM results in a decrease in amplitude and frequency (17 cells), suggesting that Ca^{+2} may to some extent block the subsynaptic Na^+ channel. Increasing the K^+ concentration from 2 to 5 mM results in a 100 fold increase in the EPSP frequency with no change in amplitude (24 cells), while lowering the K^+ concentration to 0 mM decreases release (4 cells), confirming that the hair cell release process is affected by presumed small changes in its membrane potential. These findings suggest that the subsynaptic channel may resemble that found at the neuromuscular junction and indicate that this neuropil is very sensitive to small changes in ionic concentrations.

Supported by NSF (BNS 86-16738) and NASA (NAG 2-498).

401.6

NUCLEUS OF THE OPTIC TRACT (NOT); EFFECTS OF MUSCIMOL ON GENERATION OF OPTOKINETIC NYSTAGMUS (OKN), AND SUPPRESSION AND HABITUATION OF VESTIBULAR NYSTAGMUS. H. Reisine, B. Cohen. Dept. of Neurol., Mt. Sinai Sch. of Med., NY 10029.

Single unit studies indicate that NOT processes visual motion signals that can produce ipsilateral slow phases of nystagmus (Hoffmann et al.,1986; Mustari & Fuchs, 1989). Stimulation and lesions studies further indicate that NOT activates the velocity storage mechanism in the vestibular system to produce the slow component of horizontal OKN and OKAN (Kato et al.,1988; Schiff et al., 1988, 1990). In this study we injected the GABAa agonist, muscimol, into NOT of monkeys. They developed spontaneous nystagmus with contralateral slow phases and lost OKN and OKAN with ipsilateral slow phases. Per- and post-rotatory nystagmus and OKAN with contralateral slow phases were substantially prolonged, and all previous habituation of contralateral nystagmus was lost. Slow phases to the contralateral side, whether induced during OKN or per- or post-rotatory nystagmus, could not be suppressed by exposure to a stationary visual surround. Suppression of vestibular nystagmus with ipsilateral slow phases was unaffected. These changes lasted only for the period after injection, and the animals had recovered by 24 hours later. These data suggest that activity responsible for visual suppression and for habituation of contralateral slow phases of nystagmus whether induced by visual or vestibular stimulation, is mediated through NOT. Since lesion of the nodulus of the cerebellum reduces suppression and habituation of nystagmus in ways similar to the results of muscimol injections a NOT-nodulus link is proposed for the control of these behaviors. Supported by EY02296 and EY01867.

401.7

COMPENSATION FOR HEMILABYRINTHECTOMY IN A RECURRENT NEURAL NETWORK MODEL OF THE VESTIBULO-OCULAR REFLEX (VOR). T.J. Anastasio, Dept. of Otolaryngology, University of Southern California, Los Angeles, CA 90033.

Velocity storage is permanently lost following compensation for hemilabyrinthectomy. To gain insight into the possible mechanism of this loss, compensation was simulated as learning in a recurrent neural network model of the VOR. The three network layers represented horizontal canal afferents, vestibular nucleus neurons (VNNs) and eye muscle motoneurons. Non-vestibular inputs were included. The network produced velocity storage via inhibitory commissural loops (CLs) between some VNNs.

Removal of the left canal input decreased VOR gain and unbalanced the network. Left VNNs *inside* CLs were driven into cut-off by the combined loss of canal excitation and increased commissural inhibition. This broke the CLs and eliminated velocity storage. Compensation was due primarily to increases in non-vestibular inputs. VNNs *outside* CLs regained their spontaneous rates, restoring balance and then VOR gain. Loss of velocity storage resulted from failure of VNNs *inside* CLs to also regain their spontaneous rates.

401.8

IN VITRO AND IN VIVO STUDIES OF THE HISTAMINERGIC RECEPTORS IN THE MEDIAL VESTIBULAR NUCLEI NEURONES OF THE GUINEA-PIG.

M. SERAFIN, A. KHATEB, C. de WAELE*, N. VIBERT, P.P. VIDAL* AND M. MUHLETHALER, *Dept de Physiologie, CMU, 1211 Genève 4, Switzerland and *Laboratoire de Physiologie Neurosensorielle, CNRS, Paris, France.*

It has recently been shown that a direct pathway links the tuberomammilary histaminergic neurones with the vestibular complex. Since we have described, in vitro, two main neuronal cell types in the medial vestibular nuclei (which could correspond in vivo to the tonic and phasic neurons) we have tested their sensitivity to various agonists and antagonists of the histaminergic receptors .

Using intracellular recordings in slices we found that the bath-application of histamine induced in both neuronal subtypes a reversible increase in firing frequency. This effect was accompanied by a small membrane depolarization with no (or only minor decrease) change in membrane input resistance. In both cell types, the effect was mimicked by Impromidine (a specific H2 agonist). When tested, Cimetidine (a H2 antagonist) was able to block the excitatory effect of histamine. Parallel studies performed in vivo with IP and local perfusion of the vestibular nuclei confirmed the functional implications of histaminergic receptors in the vestibular control of gaze and posture. Given that antihistaminergic drugs are widely used in the treatment of various vestibular syndromes including motion and space sickness, this model could be useful to investigate new therapeutics.

(Supported by a Swiss NSF grant no. 31-26495.89 and the french Ministère des Affaires Etrangères).

401.9

ACTION OF THE OCCUPATIONAL HAZARDS ON THE VESTIBULAR SYSTEM. K.F.Trinus. BCA Foundation. 27 Lukianivsky Str., apt.47, Kiev-71, Ukraina, GSP 252601.

A total number of 344 persons were studied, 29 of them were control healthy group, 273 - stuff of the electric power station, 27-tractor drivers, 15-welders. For examination Uemura's, Fukudas', pointing tests and oculography were used. Vestibular evoked potentials were recorded in the control group and in tractor drivers. 93% of drivers complainted vertigo. In this group were also found the disturbances in the performance of all the traditional clinical tests. On the oculograms were seen the low frequency components, which increased after the lateral gaze manoeuvres. The vestibular evoked potentials in this group had significantly increased latencies of the peaks. The welders had no signs of the vestibular damage. The oculogram had typical low frequency components, disappearing after the lateral gaze manoeuvre. Out of the electric power station stuff only 6.6% had vestibular impairment, 31.5% had mildly expressed changes of the vestibular function. The most important changes were seen due to the Uemura's and Fukudas' tests.

NEUROGLIA AND MYELIN III

402.1

CIS-ACTING REGULATORY ELEMENTS FOR MBP TRANSCRIPTION IN MYELINATING CELLS. L.G. Wrabetz*, S. Shumas*, H. Vogelbacker*, J. Grinspan, D. Pleasure, and J. Kamholz. Department of Neurology, University of Pennsylvania School of Medicine, Philadelphia, PA 19104

Characterizing the regulatory network of cis- and trans-acting factors necessary to effect the coordinate program of myelin-specific gene expression is central to understanding the regulation of myelin synthesis and assembly during development and remyelination. In order to identify the functional cis-acting elements necessary for tissue-specific expression of the myelin basic protein (MBP) gene expressed in both oligodendrocytes (OL) and Schwann cells (SC), we transfected MBP promoter-CAT (chloramphenicol acetyl transferase) fusion constructs into OL and SC. We have found that the 150 base pair region upstream of the MBP gene contains sequences which are sufficient to promote CAT expression in a tissue-specific and orientation-dependent manner in both cell types.

We have extended this analysis and have found that the start site of MBP transcription, as determined by primer extension and RNAse protection studies, is identical in OL and SC, suggesting that the MBP promoter region should be the same in both cell types. We have also found that the cap site of the MBP promoter-CAT fusion transcript is identical to that of the endogenous MBP message. Transfection of SC with a series of MBP promter-fusion constructs, containing overlapping deletions within the 150 bp region sufficient for tissue specific CAT expression, demonstrated that the 5' boundary of a tissue-specific cis-acting element lies between nucleotides -150 and -100. We are now carrying out similar transfection studies in order to identify the 5' boundary of the tissue-specific element(s) used in OL.

402.2

DIFFERENTIAL PROLIFERATIVE RESPONSE OF ADULT HUMAN AND NEONATAL MOUSE ASTROCYTES TO CYTOKINES. R. Moumdjian*, V.W. Yong and J.P. Antel. Neuroimmunology Unit, MNI, McGill University, Montreal, Quebec H3A 2B4, Canada.

In response to most forms of injury to the adult CNS, astrocytes undergo hypertrophy and/or hyperplasia. We have targeted cytokines as possible proliferative factors for astrocytes because infiltration of mononuclear cells is commonly seen after many types of CNS injury. Using a double immunofluorescence GFAP-BrdU technique (Yong and Kim, J. Neurosci. Methods, 21:9,1987), we have identified the following as mitogens for cultured human adult astrocytes: supernatants from activated CD8+ or CD4+ human lymphocytes (proliferation index of 22.2 ± 2.3 and 16.5 ± 2.5), γ-interferon (6.5 ± 1.1), and interleukin-1 (6.2 ± 0.7). In agreement with these in-vitro results, γ-interferon when applied in vivo increased the extent of reactive gliosis in adult injured mouse brain. When cultured neonatal mouse astrocytes were treated, there was either no alteration (for interleukin-1), or a decrease in proliferation was observed (activated CD8+ supernatant: 40% of controls; γ-interferon: 60% of controls). We suggest that the intrinsic proliferative capability of astrocytes (low for adult human and high for neonatal mouse) determines the mitotic response to cytokines: proliferation for adult astrocytes, and differentiation/maturation with subsequent decrease in proliferation for neonatal astrocytes.

402.3

INVOLVEMENT OF PROTEIN KINASE C IN PROLIFERATION OF ASTROCYTES. V.W. Yong. Neuroimmunology Unit, MNI, McGill University, Montreal, Quebec H3A 2B4, Canada.

The objective was to assess the mechanism(s) by which proliferative agents for astrocytes exert their response. As determined using a GFAP-BrdU technique, we have identified the following cytokines as mitogens for cultured human adult astrocytes: supernatants from activated CD8+ human lymphocytes, γ-interferon and interleukin-1. For neonatal mouse astrocytes, rather than the above cytokines, fibroblast growth factor (FGF, 100 ng/ml) increased proliferation (to 250% of controls). To assess the mechanism of proliferation, we targeted the enzyme protein kinase C (PKC) because pharmacological activators of this enzyme (phorbol esters) produced proliferation of both human adult and mouse neonatal astrocytes. For human adult astrocytes, H-7 (a relatively selective inhibitor of PKC) inhibited the response of all mitogenic cytokines; 10 μM H-7 reduced proliferation by 50%, while 50 and 100 μM H-7 completely inhibited mitosis. For neonatal mouse astrocytes, H-7 in a dose-dependent manner reduced the basal proliferation, and also inhibited the mitogenic effect of FGF. ATP and dbcAMP (PKA system) and the calcium ionophore A23187 did not significantly alter the proliferation of astrocytes. Thus, the enzyme PKC appears to be a common signal transduction pathway for agents identified to be mitogens for human adult and mouse neonatal astrocytes.

402.4

CELL CYCLE-SPECIFIC REQUIREMENT FOR MEVALONATE IN ASTROCYTES FROM LONG TERM PRIMARY CULTURES Thomas J. Langan and Mary Slater*, Dept. of Neurology, S.U.N.Y. at Buffalo School of Medicine, Buffalo N.Y. 14222

Astrocytes retain the capacity to divide after completion of brain maturation. We hypothesized that astrocytes in primary cultures can be stimulated to re-enter the cell division cycle after prolonged quiescence, and that mevalonate, the precursor of isoprenoid lipids, regulates this process. DNA synthesis (uptake of [3H] thymidine into acid-precipitated material) declined by 80±9% with confluence in cultures of newborn rat brains in 10% calf serum (CS). After 2, 6, and 16 weeks the cultures were trypsinized and replated at 10^4 cells/cm^2. 72h later, 79±9% of cells were astrocytes by glial fibrillary acidic protein immunofluorescence. Serum shift-down (10% to 0.1%) for 48h followed by re-exposure to 10% CS resulted in 12h of quiescence (G0+G1), then in a 6-fold increase (263±34 vs. 43±6 CPM/ug. prot./h) in DNA synthesis (S phase). This increase was abolished by addition of mevinolin, a competitive inhibitor of mevalonate synthesis, and mevalonate reversed this block of DNA synthesis when added as late as 12h after serum stimulation. Cholesterol-rich lipoproteins failed to reverse the inhibition of DNA synthesis by mevinolin.

Thus, astrocytes after several months in primary cultures: 1. can be stimulated to re-enter the cell cycle; 2. require mevalonate or a nonsterol derivative thereof in late G1.

402.5

ALTERATIONS IN Na^+-CURRENT CHARACTERISTICS DURING DEVELOPMENT OF HIPPOCAMPAL ASTROCYTES *IN VITRO*.

H. Sontheimer, B.R. Ransom, A.H. Cornell-Bell, J.A. Black and S.G. Waxman. Dep. Neurology, Yale Univ., New Haven, CT 06510.

Na^+-current expression in hippocampal astrocytes was studied during the first 20 days *in vitro* (DIV) using whole-cell patch-clamp. The percentage of astrocytes expressing Na^+-currents decreased from 75% at 1 DIV to about 30% after 10-20 DIV. Na^+-currents could be differentiated into two types: a current with fast activation and inactivation and an h_∞-curve with a midpoint around -65mV and a second type with slower kinetics and an h_∞ midpoint around -85mV. The first type of Na^+-current was seen 1-5 DIV, the second type after 6-20 DIV. Current-voltage curves of Na^+-current activation were identical in astrocytes of all ages. Na^+-current densities displayed a biphasic time-course with an initial loss during the first 5 DIV, followed by re-expression. This time-course mirrored the change from the first (midpoint -65mV) to the second type of Na^+-current (-85mV), the latter only expressed later than 5 DIV. Interestingly Na^+-current expression was restricted almost exclusively to astrocytes that were not dye-coupled as assayed by following the spread of Lucifer Yellow. Transiently uncoupling cells with octanol did not unmask Na^+-currents. These results demonstrate that two types of Na^+-currents are expressed in hippocampal astrocytes *in vitro*, and suggest a relationship between coupling and Na^+-channel expression.

402.6

ACTIVATION OF ENDOTHELIN RECEPTORS REGULATES INTRACELLULAR CA^{2+} IN CULTURED CEREBELLAR ASTROCYTES J.A. Holzwarth, S.R. Glaum and R.J. Miller. The Dept. of Pharm. and Physiol. Sciences, The University of Chicago, 947 E 58th Street, Chicago, Il 60637

Sarafotoxins (SfTx) are vasoconstrictive peptides present in snake venom that act at vascular smooth muscle endothelin receptors. SfTx has been shown to induce changes in PI turnover in rat brain, endothelin binding being highest in the cerebellum. We studied the effects of SfTx on $[Ca^{2+}]_i$ in single astrocytes using fura2 based microspectrofluorimetry and digital imaging. SfTx caused increases in $[Ca^{2+}]_i$ in astrocytes cultured from cortex, hippocampus and cerebellum. The response was examined further in cerebellar astrocytes. Perfusion with SfTx (1nM) in Ca^{2+} free medium (with 20uM EGTA) caused a spike of intracellular Ca^{2+}. In medium containing Ca^{2+}, this spike was followed by a plateau. The plateau, not the spike, could be abolished by 1mM Ni^{2+}. However, in the majority of astrocytes the plateau was not blocked by nimodipine (10uM). Neither the spike nor the plateau were blocked by pre-incubation with pertussis toxin (16hr, 250ng/ml). In contrast the spike was blocked by phorbol ester treatment (10min, 1uM PdBu). The response to SfTx desensitized at high concentrations (100nM), but did not do so at low concentrations (1nM). Following the wash of SfTx, the $[Ca^{2+}]_i$ plateau declined rapidly in some cells but in other cells persisted for many minutes. In these cases, removal of extracellular Ca^{2+} caused $[Ca^{2+}]_i$ to decline but the plateau was formed again on reintroduction of extracellular Ca^{2+}. Thus, SfTx in addition to mobilizing $[Ca^{2+}]_i$, appears to activate a Ca^{2+} permeable channel, that may be similar to that described in other cells following elevation of IP_3.

402.7

CALCIUM WAVES PROPAGATE THROUGH ASTROCYTE NETWORKS IN DEVELOPING HIPPOCAMPAL BRAIN SLICES. John W. Dani, Alex Chernjavsky* & Stephen J Smith*. *Molecular and Cellular Physiology, Stanford University School of Medicine, Stanford, CA 94305.*

Using a laser confocal microscope and the calcium indicator dye fluo-3, we have detected waves of elevated intracellular Ca concentration that propagate through slices of neonatal rat hippocampus. At 20 degrees C, these waves propagate over distances of 1 mm or more at velocities of 10-20 micrometers per second. Because these Ca waves closely resemble those recently described in pure cultures of hippocampal astrocytes (Cornell-Bell, et al., 1990, Science 247: 470-3), we suggest that the waves observed in hippocampal slices also propagate through coupled networks of astrocytes. Ca waves can be observed in resting, unstimulated slices, but increase greatly in amplitude and extent when neurons within the slice are stimulated using N-methyl-D-aspartate (NMDA). Based on earlier observations that NMDA does not induce Ca waves in pure cultures of astrocytes, we propose that NMDA directly stimulates only the neurons within our hippocampal slices, and that the observed enhancement of the putative glial Ca waves by NMDA therefore refelects a form of prompt neural-glial communication, perhaps mediated by neuronal release of glutamate, a compound which does stimulate Ca waves in pure astrocyte cultures. These findings suggest that astrocyte networks may play a greater role than previously recognized in neural computation and long-distance signalling within the brain. *Supported by the G. Harold and Leila Y. Mathers Charitable Foundation.*

402.8

A GLIAL CYTOCAL WAVE IS THE CONDUCTION VELOCITY-DETERMINING PROPAGATION MECHANISM OF SPREADING DEPRESSION. D. H. Leibowitz. CNS Res. Fndtn, Inc., N.Y., NY 10025.

The propagation mechanism of Leão's spreading depression (SD), which plays a role in the pathophysiology of migraine, remains enigmatic. Through a program of theoretical research I have now identified the probable mechanism. (1) A detailed analysis of the literature reveals that the fundamental propagation mechanism of SD is probably located in the glial compartment and has the nature of an extraordinarily slow, metabolically-controlled glial action potential, which helps trigger the neuronal depolarization of SD and is in turn facilitated by neurotransmitter-released agents. (2) There exists among eukaryotes a widespread class of intracellular waves of excitation apparently based upon the Ca^{++}-induced release of Ca^{++} from intracellular stores combined with cytoplasmic diffusion of Ca^{++} (cytocal waves). The propagation velocity of SD is typical of a cytocal wave. The Arrhenius activation energy of the conduction velocity rate-limiting mechanism of SD is typical of a cytocal wave. The tendency of SD to occur under stressful or injurious conditions is common among cytocal waves. The effect of an imposed constant electric field on the propagation velocity of SD is the effect expected on a cytocal wave. On the basis of these and many other arguments I propose with a high degree of confidence that a neuroglial cytocal wave is the conduction velocity-determining mechanism of SD.

402.9

ULTRASTRUCTURAL IDENTIFICATION OF HRP INJECTED OLIGODENDROCYTES IN THE INTACT RAT OPTIC NERVE B.R. Ransom, A.M. Butt* and J.A. Black, Dept. Neurology, Yale Univ. Sch. Med., New Haven, CT, 06510.

The three-dimensional morphology of mammalian oligodendrocytes is not well defined; these cells are not reliably stained by Golgi impregnations and they are difficult to reconstruct from EM images because the tenuous connections between parent oligodendrocytes and their myelin segments are difficult to follow in serial sections. In a preliminary report (A. Butt and B. Ransom, Glia 2:470-475, 1989) we described the morpholgy of cells presumed to be oligodendrocytes in the intact rat optic nerve (RON) using intracellular injections of horseradish peroxidase (HRP); these cells had 10-20 longitudinal processes, each 200-300 µm long, that were oriented along the long axis of the RON parallel to the axons. The longitudinal processes of these cells were connected near their midpoints to the cell body by thin branches, 15-30 µm long. By EM examination of such HRP-filled cells within the RON, we have now directly determined their identity. Glial cells in 12-30 postnatal day optic nerves were filled with HRP and reacted using standard techniques. Three nerves containing isolated HRP injected cells with the morphology described above, were serially sectioned for viewing with transmission EM. HRP was found in paranodal loops at nodes of Ranvier and in the inner and outer tongue processes of myelin sheaths, as well as in cell bodies. This distribution of HRP clearly identified these cells as myelin-producing oligodendrocytes. The longitudinal processes of these cells, as seen with light microscopy, correspond to the HRP-filled tongue processes and therefore define the number (i.e., 10-20) and length (i.e., 200-300 µm) of the internodal myelin sheaths provided by individual oligodendrocytes. Since the entire cytoplasmic border surrounding the myelin sheath readily filled with HRP, this compartment could serve as an efficient conduit for metabolites or membrane constituents. Supported by NIH grant NS 15589.

402.10

INDUCIBLE EXPRESSION OF A HYBRID GENE IN TRANSGENIC MICE MARKS A SUBGROUP OF ASTROCYTES. L.Mucke, J.C.Morris*, M.B.Oldstone*, M.I.Nerenberg*. Div.of Virology, Dept.of Neuropharmacology, Research Institut of Scripps Clinic, La Jolla, CA 92037.

A system was developed that allows the expression of hybrid genes in astrocytes in vivo. Almost the entire murine glial fibrillary acidic protein (GFAP) gene was incorporated in the construct. The lacZ reporter gene was inserted into the GFAP cassette and injected into the germline of mice. B-gal assays and anti-GFAP Abs revealed a similar expression pattern in brain sections from different transgenic lines. GFAP-lacZ expression was limited to GFAP-positive cells but not all GFAP-positive cells expressed GFAP-lacZ. Focal cerebral lesions induced GFAP-lacZ expression in regions where the transgene was not expressed at baseline. Hence the GFAP construct allows expression of hybrid genes in astrocytes in vivo. Inclusion of all regulatory elements of the GFAP gene into the GFAP cassette restricts expression of hybrid genes to cells that express endogeneous GFAP and allows induction of hybrid gene expression by gliosis-provoking maneuvers. GFAP-lacZ expression identifies a subgroup of astrocytes and can be used as a marker of astrocyte activation.

402.11

INTRACELLULAR pH REGULATION IN ASTROCYTES IN THE ABSENCE OF HCO_3^-. W.F. Boron*, G. Boyarsky, K.L.Marek, and B.R. Ransom, Depts. of Cell. and Mol. Physiol. and Neurol., Yale U. Sch. of Med., New Haven, CT 06510.

Intracellular pH (pH_i) was measured microfluorometrically in single astrocytes cultured from neonatal rat forebrain, using the pH-sensitive dye BCECF. In many cell types, brief exposure to NH_4^+/NH_3 (NH_4^+) causes an abrupt acidification, recovery from which can be used as an assay for mechanisms responsible for pH_i regulation. In astrocytes, we now report that application of NH_4^+ (1-20 mM) causes a tetraphasic change in pH_i. For example, in five cells 5 mM NH_4^+/NH_3 causes: (1) a rapid increase from 6.90 ± 0.03 to 7.23 ± 0.03, presumably due to NH_3 entry, (2) a subsequent slow decline to 7.03 ± 0.02, perhaps due to NH_4^+ entry, (3) a slower increase to 7.29 ± 0.05, and finally (4) a subsequent decline to a new stable level of 6.88 ± 0.04. The subsequent removal of NH_4^+ causes an abrupt acidification from which pH_i rapidly recovers. However, the rate of recovery at any pH_i does not depend uniquely on that pH_i but on the history of the experiment (e.g., the recovery is slower when the pH_i goes to very acid levels following NH_4^+ removal). In contrast, brief removal of external Na^+ (~5 min) causes a monotonic fall in pH_i from 6.88 ± 0.02 to 6.36 ± 0.02, with little pH_i recovery. Readdition of Na^+ leads to a rapid pH_i recovery. The recovery rate at any pH_i depends only on that pH_i, and not on the initial pH_i or the degree to which the pH_i fell during the period of Na^+ removal. This recovery rate is inhibited ~40% by brief removal of external Cl^-, ~50% by pretreatment with 50 µM DIDS, and ~75% by Cl^- removal and DIDS-pretreatment. The Na^+-, Cl^-- and DIDS-sensitivities of the recovery suggests that a Na^+-dependent Cl-HCO_3 exchanger could be operating in the nominal absence of HCO_3^-, or that Cl^- removal or DIDS could affect a Na^+-dependent, HCO_3^--independent transport process.

402.12

Do Oligodendrocyte Precursor Cells Persist in Adult Human CNS? R. Armstrong*, H. Dorn*, C. V. Kufta*, and M. Dubois-Dalcq, NINDS, NIH, Bethesda, MD 20892

Recent studies indicate that adult rodent CNS contains an oligodendrocyte precursor cell which expresses glycolipid antigens recognized by the O4 antibody. To investigate whether this cell type may be present in adult human CNS, we cultured temporal lobe tissue which was resected during treatment of intractable epilepsy. Optimal cultures of oligodendrocyte lineage cells were prepared from initially non-adherent cells which were reseeded after 2 days. Using 3-color immunofluorescence, we consistently identify a population of putative oligodendrocyte precursor cells which express antigens recognized by O4 but do not express a differentiation marker of oligodendrocytes (galactocerebroside, GC) or astrocytes (glial fibrillary acidic protein, GFAP). The number of O4 only cells was highest during the first week in vitro and decreased over the following weeks. In the same cultures the number of oligodendrocytes (GC+ O4+) either remained constant or increased in defined media conditioned by rat glial cells. Relatively few type 2 astrocytes (O4+ GFAP+) were present in cultures grown in defined media or media containing 5% fetal bovine serum. In the conditions tested, oligodendrocytes and O4 only cells did not incorporate 3H-thymidine during a 3d pulse even though some type 2 astrocytes were labeled.

Thus, adult human CNS may harbor "resting" oligodendrocyte precursor cells with an antigenic phenotype similar to that seen in the rodent. Further studies are required to determine the proliferation and/or differentiation potential of these cells and discern whether glial cells with a similar phenotype can be identified in vivo.

NEURAL-IMMUNE INTERACTIONS I

403.1

LOCALIZATION OF INTERLEUKIN-1 mRNA IN RAT BRAIN. J. Licinio, M.-L. Wong, M.A. Smith, P. W. Gold*. Clinical Neuroendocrinology Branch, National Institute of Mental Health Intramural Research Program, Bethesda, MD 20892.

Neurons containing interleukin-1 (IL-1) have been identified in human hypothalamus by immunohistochemistry (Breder CD *et al.*, Science 1988; 240:321). IL-1 affects the release of hypothalamic neuropeptides and has multiple physiologic effects. In the present study we have further characterized IL-1-producing brain regions by determining the localization of cells containing IL-1 mRNA. We have used *in situ* hybridization histochemistry to determine the anatomic distribution of brain IL-1 mRNA. *In situ* hybridization was done with ^{35}S-dATP labelled oligodeoxynucleotide probes. Sections were apposed to films and dipped in NT-2 emulsion (Kodak). Our results indicate that IL-1 is synthesized throughout the brain, especially in white matter. Given the potent effects of IL-1 on brain function and behavior, these data raise the possibility that white-matter cytokines may modulate brain function. Further studies are necessary to clarify the physiologic effects of endogenous brain IL-1.

403.2

INTERLEUKIN INDUCES RELEASE OF DOPAMINE AND ITS METABOLITE FROM THE HYPOTHALAMUS *IN VIVO*. P. S. Mohankumar*, S. Thyagarajan* and S.K. Quadri, Neuroendocrine Research Laboratory, Kansas State University, Manhattan, KS-66506.

Interleukin-1B (IL-1B), a lymphokine, stimulates the release of hypothalamic and pituitary hormones. The mechanism of this action is not clear. In this report we present evidence for a mechanism of IL-1B effects on the hypothalamus and pituitary. Push-pull cannulae were implanted stereotaxically in the ventromedial nucleus of the hypothalamus of 8-month old Sprague-Dawley male rats. The hypothalamic nuclei were perfused with 100 ng or 50 ng of IL-1B or its vehicle (20 ul artificial cerebrospinal fluid). The perfusate samples were analyzed for catecholamines by electrochemical high performance liquid chromatography. The higher dose (100 ng) of IL-1B produced an increase of more than 100% in dopamine (DA) release within 50 minutes of perfusion. DA release continued to increase and was about 120% by 100 minutes post-perfusion. The lower dose of 50 ng IL-1B also increased DA release but it did not reach statistical significance. There was no significant change in DA release in control rats. Similarly, 100 ng IL-1B produced a significant increase in the release of 3,4-dihydroxyphenyl acetic acid (DOPAC). The lower dose of 50 ng IL-1B also increased DOPAC release, but it was not significant. It is concluded that IL-1B affects the release of hypothalamic and pituitary hormones by increasing the release of catecholamines from specific hypothalamic nuclei. To our knowledge this is the first evidence for a direct action of IL-1B on neurotransmitter metabolism in the brain *in vivo*.

403.3

INTERLEUKIN-1 (IL-1) AUGMENTS GABA-DEPENDENT CHLORIDE UPTAKE IN CORTICAL SYNAPTONEUROSOMES. L.G. Miller, W.R. Galpern, M. Lumpkin*, S. F. Chesley*, C.A. Dinarello*. Div. of Clinical Pharmacology, Depts. of Psychiatry and Pharmacology, and Dept. of Medicine, Tufts-New England Med. Ctr., Boston, MA 02111

The cytokine IL-1, a major participant in inflammatory and immune events, is present in the CNS and specific receptors appear to exist in brain. To assess a possible interaction between IL-1 and the GABAergic system, we evaluated effects of IL-1 on GABA-dependent chloride uptake in mouse cortical synaptoneurosomes. Uptake was determined using the GABA analog muscimol over a 6 sec interval. Preincubation of synaptoneurosomes (10 min) with IL-1, 100 pg-10 ng/ml, augmented maximal chloride uptake by approximately 40%. There was no significant alteration in the EC_{50} for muscimol with IL-1. At both higher and lower concentrations of IL-1, there were no significant effects on GABA-dependent chloride uptake. Addition of IL-1 concurrently with muscimol had a smaller, but still significant increase in chloride uptake at IL-1 concentrations of 100 pg/ml and 1 ng/ml. A specific IL-1 receptor antagonist, 10 ng/ml, prevented the effect of 100 pg/ml or 1 ng/ml IL-1 on maximal chloride uptake. These data indicate that IL-1, acting at a specific binding site in brain, augments GABA-dependent chloride uptake in cortical membrane preparations.

403.4

ENDOTOXIN TOLERANCE FAILS TO MODIFY CYTOKINE STIMULATED NOREPINEPHRINE TURNOVER IN MOUSE HYPOTHALAMUS. I. N. Mefford, C. F. Masters*, M. P. Heyes and R. L. Eskay*. Sec. Clin. Pharmacol., Clin. Neurosc. Branch, NIMH, Bethesda, MD 20892

Acute administration of endotoxin (lipopolysaccharide, LPS) as well as interleukin-1 (IL-1) stimulate hypothalamic norepinephrine turnover by a prostaglandin dependent mechanism. The rapid adaptation to the physiological effects of endotoxin is well known as endotoxin tolerance. The neurochemical consequences of chronic endotoxin administration have not been studied. We administered LPS or saline to male C57BL mice (5 ug/mouse/day, i.p.) for 7 days. On day 8, animals were challenged with saline, LPS (5 ug/mouse), IL-1 (1 ug/mouse) and tumor necrosis factor (TNF, 1 ug/mouse) and decapitated after two hours. Blood was collected for corticosterone and brain parts dissected for determination of catecholamines, serotonin and metabolites. The stimulation of hypothalamic norepinephrine turnover observed after acute LPS administration was absent following chronic LPS administration. However, the acute LPS stimulated elevation of corticosterone was only partially attenuated. The acute IL-1 and TNF-stimulated increase in hypothalamic norepinephrine turnover as well as the cytokine-induced elevation of corticosterone were unaffected by chronic administration of LPS. These results suggest that endotoxin desensitization occurs at the level of the macrophage in response to endotoxin. Other neuroendocrine, cytokine-mediated responses appear to be unaffected following desensitization of the macrophage response to endotoxin.

403.5

INTERLEUKIN 1 INDUCES c-FOS and c-JUN m-RNA EXPRESSION IN AtT-20 CELLS BY A MECHANISM INDEPENDENT OF PROTEIN KINASE C AND PROTEIN KINASE A. Mirela O.Fagarasan*, Katherin Muegge+,Francesca Aiello+, S.K. Durum+and J. Axelrod* (SPON: J.Axelrod) *National Institute of Mental Health, Bethesda, M.D. and +National Cancer Institute, Frederick, MD.

We have demonstrated that IL-1 after a long period of treatment direcly stimulates β-endorphin release and potentiates the effects of other secretagogues in AtT-20 cells, a mouse anterior pituitary cell line (Fagarasan et al., PNAS, 86, 2070-2073). It was also observed that IL-1 markedly phosphorylated 19-, 20-, and 60 kDa proteins (Fagarasan et al, PNAS, 87, 2556-2559). We found that another early signal triggered by IL-1 in AtT-20 cells involves the enhancement of c-fos and c-jun mRNA expression. The effect appeared within 30 minutes, is transient and returned to basal levels after 2hr. Desensitization of protein kinase C by phorbol ester (TPA) pretreatment had no effect on the ability of IL-1 to induce c-fos and c-jun m-RNA expression. An experiment was designed to investigate whether IL-1 can still generate this early signal after its continous presence with AtT-20 cells for 24 hours. After prolonged treatment with IL-1, the capacity of IL-1 to induce c-fos mRNA expression was abolished, but TPA and CRF effect on c-fos mRNA was markedly expressed.
Whether the interaction of these intermediate early gene products is involved in IL-1 potentiation of B-endorphin secretion induced by secretagogues awaits further investigation.

403.6

SUPPRESSION OF NATURAL KILLER CELL ACTIVITY FOLLOWING ELECTRICAL STIMULATION OF THE RAT MESENCEPHALON. R.J. Weber and A. Pert. Neuroimmunology Unit, Laboratory of Medicinal Chemistry, NIDDK, NIH, and Biological Psychiatry Branch, NIMH, Bethesda, MD 20892

We have previously reported that microinjections of morphine into the periaqueductal gray matter (PAG) of the mesencephalon produce suppression of natural killer cell (NK) activity (Weber and Pert, *Science* : 245: 188, 1989). Electrical stimulation of the periventricular region and the PAG have also been shown to produce opioid-like effects in the rat. The purpose of this study was to determine whether electrical stimulation of the PAG would also suppress NK cell activity. Rats were implanted with bipolar electrodes aimed for various rostral and caudal planes of the PAG. One week following recovery the animals were stimulated with intermittent 150 msec trains of biphasic pulses (50usec in duration presented at 100HZ and 500uA) for 20 minutes. Splenic NK cell activity was determined 3 hours following termination of stimulation. The most active sites in suppressing NK cell activity were found in the caudal PAG, just lateral to the dorsal raphe. Injections of morphine into a similar region were also found to suppress NK cell activity in the previous study cited above. Additional sites that were involved in alteration of NK cell activity were found in the caudal periventricular gray matter extending from the level of the posterior hypothalamus to the red nucleus. Studies are underway to determine the neural circuitry through which these signals gain access to the immune system.

403.7

CALCITONIN GENE RELATED PEPTIDE (CGRP) AND ITS RECEPTORS IN THE MOUSE THYMUS AND SPLEEN. K. Bulloch, J. Hausman*, T. Melnechuk, T. Radojcic*, S. Short*, D.M. Simmons, L.W. Swanson. Dept. of Psychiatry, Univ. of Cal. San Diego, La Jolla, CA 92093; Salk Institute, San Diego, CA 92138

In this study we demonstrate with immunocytochemistry the presence of CGRP in intrathymic mast cells, nerves and a discrete population of cells at the corticomedullary boundaries. In situ hybridization confirms that the subcapsular and trabecular mast cells and the intrathymic cells at the corticomedullary boundary synthesize messenger RNA for CGRP. Little to no CGRP activity was observed in the nerves or cells of the spleen. However, receptor sites for CGRP can be detected in both spleen and thymus tissue. These sites bind iodinated CGRP with a Kd of 14nM (Bmax 8 fmol/mg protein) for spleen and 12nM (Bmax of 18 fmol/mg protein) for thymus. These data indicate that CGRP may play a role in lymphocytic immune interactions. Supported by ONR grant #N0014-89-J-1256.

403.8

REGULATION OF PRE-PROENKEPHALIN A (PPEA) GENE EXPRESSION IN NORMAL MURINE T LYMPHOCYTES. K.M. Linner,* S. Nicol and B.M. Sharp*. Minneapolis Medical Research Foundation and Departments of Medicine, Hennepin County Medical Center and University of Minnesota, Minneapolis, MN 55404

Recent studies have shown that cells of the immune system can express genes for endogenous opioids. For example, PPEA mRNA has been found in transformed T cell lines, transformed and normal murine macrophages and mast cells, and concanavalin A (Con-A) - stimulated murine T helper cell clones. Our studies extend these findings regarding PPEA gene expression to normal murine thymus and spleen. Thymocytes and splenocytes isolated from female CD-1, SPF mice (4-5 w.o.) were cultured with several doses of Con-A for 24, 48, and 72 hr. Poly A mRNA was isolated and either dot blot or Northern gel analysis was done. PPEA mRNA was identified by hybridization with a specific 50-mer 32P-cDNA. Only thymocytes were induced to express PPEA mRNA under these conditions, with peak response (15x baseline) occurring after 72 hr in culture at Con-A doses of 5-7.5 µg/ml. No PPEA mRNA was detected in thymocytes after 24 hr in culture, whereas low levels were evident at 48 hr. Two hybridizable bands were found in cultured cells, one corresponding in MW to pituitary PPEA (1400d) and one larger. No PPEA mRNA was detected in splenocytes at any time in response to any dose of Con-A , even though these cells, like thymocytes, were induced to proliferate by Con-A. PPEA mRNA expression was further shown to be inducible in CD4+ thymocytes and its expression was inhibited by IL-1-beta. A role for enkephalin peptides in the ontogeny of T cells in the thymus is postulated. (Supported by DA04196)

403.9

CORTICOTROPIN-RELEASING HORMONE (CRH)BINDING SITES ON HUMAN IM-9 LYMPHOBLASTS J.P. McGillis, S. Humphreys*, and S. Reid* Dept. Microbiology and Immunology, Univ. Kentucky Coll. Med., Lexington, KY 40536

CRH stimulates rat B cell proliferation in vitro at nM concentrations (McGillis, et al., J. Neurosci. Res. 23:346, 1989) and CRH binding sites are present in spleen membranes (Dave, et al., Endo. 116: 2152 1985; Webster and De Souza, Endo. 122:609 1988). These data suggest an immunomodulatory role for CRH in peripheral tissues. CRH binding sites on B cells were characterized on human IM-9 cells. Binding studies were done with intact cells using ^{125}I-[Tyr°]-rat/human CRH. Binding was saturable, specific, and time and temperature dependent. Specific binding had a linear dependence on cell concentration at cell densities ranging from 2.5 to 20 x 10^6 cells/ml. Equilibrium binding was dependent on temperature, with equilibrium reached at 3 hr at 27° C, and by 90 min at 37° C. No binding was observed at 4° C. Best fit analysis of saturation isotherm binding suggested a two site model, with a high affinity Kd of 17.8 ± 2.15 pM and a low affinity Kd of 2.71 ± 0.78 nM. Binding site densities were 181 ± 97 sites/cell and 4363 ± 2094 sites/cell, respectively. The presence of CRH binding sites on human B lymphoblasts supports an immunomodulatory role for CRH in peripheral tissues.

403.10

REGULATION OF PROENKEPHALIN GENE EXPRESSION IN ASTROCYTES BY CYTOKINES. K. G. Low and M. H. Melner, Divisions of Neuroscience and Reproductive Biology, Oregon Regional Primate Research Center, Beaverton, OR 97006.

Astrocytes have been implicated to play an immunological role in the brain as they express class II antigens and serve as antigen-presenting cells. In addition, astrocytes have been demonstrated to exhibit a high level of proenkephalin gene expression (Melner *et al.*, *EMBO J.*, 9:791, 1990). These observations suggest that astrocytes play a pivotal role in neuroimmune interactions. Neonatal rat astrocytes from the cerebral cortex were treated with 20 units/ml recombinant rat γ-interferon (γ-IFN) for 48 hours, 50 ng/ml recombinant human tumor necrosis factor-α (TNF-α) for the last 24 of 48 hours or both 20 units/ml γ-IFN (48 hours) and 50 ng/ml TNF-α (last 24 of 48 hours). Northern analysis using a ^{32}P-labeled 1.1 kb Eco R1/Hind III fragment of the rat proenkephalin cDNA detected a ~ 1.4 kb transcript. Proenkephalin mRNA levels appeared to be increased 2-fold with TNF-α however γ-IFN clearly decreased proenkephalin mRNA levels 2.5-fold. In addition, TNF-α had no effect on the γ-IFN inhibition of proenkephalin mRNA levels. Northern analysis with a ^{32}P-labeled 2.4 kb Eco R1 fragment of the rat glutamine synthetase cDNA detected 2.8 kb (more abundant) and 1.4 kb transcripts and demonstrated that glutamine synthetase mRNA levels were not significantly changed under the treatments with these cytokines. This inhibition specifically of neuropeptide gene expression in astrocytes by γ-interferon suggests potential interactions between the onset of viral infection of brain tissues and the subsequent effects upon neurological function. Supported in part by NIH DK41035, NIH RR-00163, and ONR N00014-90-J-1122.

403.11

INTIMATE CONTACT BETWEEN RAT BASOPHILIC LEUKEMIA (RBL) AND PHEOCHROMOCYTOMA (PC-12) CELLS COMBINED IN CULTURE. J. Solomonides*, V. Dimitriadou, M. Holdridge* and T.C. Theoharides. Department of Pharmacology, Tufts University School of Medicine, 136 Harrison Avenue, Boston, MA 02111

Mast cells are important in allergic reactions. However,they have recently been implicated in a number of neuro-endocrine disorders and have also been shown to be in close contact with neurons. Here, we investigated the possibility that cultured analogues of mast cells and neurons may establish contact *in vitro*. RBL cells, which are homologous to bone marrow-derived mast cells, and PC-12 cells, which can differentiate to neurons, were grown in Dulbecco's modified Eagle's medium with 10% fetal calf serum and either 10 U interleukin 3 (IL-3), 1μM nerve growth factor (NGF) or both. The two cell types were combined in 1:1 ratio of about 3-5x10^5 cells and were examined after 5 days. The cells were grown in culture dishes with sterile cover slips which were fixed for light, as well as scanning and transmission electron microscopy. Close contact was evident directly between the cells and between PC-12 axonal processes and RBL cells. Histamine measurements after secretion induced by the cation ionophone A23187 indicated an increase in the cultures containing both IL-3 and NGF. These results support the premise that mast cells may be innervated directly and suggest the possibility that such mast cells may also express different phenotypic and functional characteristics.

403.12

SPLENIC NOREPINEPHRINE IS DECREASED IN MRL MICE HOMOZYGOUS FOR THE *LPR* GENE. S.Y. Felten, S.M. Brenneman, J.A. Moynihan, L.J. Grota. Depts. Neurobiology & Anatomy and Psychiatry, Univ. Rochester Sch. Med., Rochester, NY 14642

The MRL mouse has been used as a murine model of systemic lupus erythematosis. The disease is the result of a single recessive gene, *lpr*. Mice homozygous for the *lpr* gene have autoimmune disease characterized by a wide spectrum of autoantibodies, a marked lymphadenopathy and splenomegaly associated with an overabundance of an abnormal T cell subset (dull Thy 1^+, Lyt 1^+), arthritis and glomerulonephritis. Fifty percent of the *lpr* animals are dead by 24 weeks of age.

Norepinephrine was measured in spleens from *lpr/lpr* mice and their congenic +/+ controls at 6, 12, 18, and 24 weeks of age and found to be lower in *lpr/lpr* mice at all ages, both when expressed as a concentration (pMoles/g wet weight) or as pMoles/whole spleen. Both measurements were necessary, since the presence of lymphadenopathy greatly increases the number of lymphocytes in the spleen.

Glyoxylic acid histofluorescence and tyrosine hydroxylase immunocytochemistry demonstrated relatively normal patterns of innervation in periarteriolar zones, but showed no indication of innervation within the areas occupied by abnormal T cells.

CELLULAR AND MOLECULAR STUDIES III

404.1

THE ROLE OF POSTSYNAPTIC ACTIVITY IN ESTABLISHING APPROPRIATE SYNAPTIC MORPHOLOGY DURING NEUROMUSCULAR DEVELOPMENT IN DROSOPHILA EMBRYOS. M.D.S. Anderson and H. Keshishian. Dept. of Biol., Yale University, New Haven, CT 06511.

Through the use of immunocytochemistry and AGTX1, a toxin isolated from the venom of the Orb Weaver spider *Araneus gemma* that blocks glutamate activity postsynaptically at the neuromuscular junction in *Drosophila*, we have shown that the early peristaltic movements in *Drosophila* embryos are mediated by glutamate and likely neurally evoked. Early growth cone contacts on the bodywall muscles in stage 16 embryos are immunoreactive for glutamate and coordinated peristaltic bodywall muscle movements begin to be visible during this stage of development. All of the coordinated muscle contractions are abolished following the microinjection of AGTX1.

It is clear that neuromuscular activity is present during late embryogenesis, when the stereotypic ending anatomies begin to be established on *Drosophila* bodywall muscles. Therefore, we sought to identify whether activity is necessary for the acquisition of appropriate synaptic morphology. During stage 17 of embryogenesis the endings on the bodywall muscles begin to resemble the stereotypic mature larval endings. We have used AGTX1 to block postsynaptic activity in stage 17 embryos, followed by anti-HRP immunocytochemistry to look at the role of postjunctional activity in the development of stereotypic ending patterns and synaptic boutons. There appears to be no effect on the establishment of normal ending morphology when the postsynaptic response is blocked. We have also begun to microinject TTX into stage 17 embryos to determine whether blocking action potentials during this stage affects the development of appropriate synaptic morphology.

404.2

ANTAGONISM OF THE NMDA RECEPTOR AT THE SPINAL SEGMENTAL LEVEL DISRUPTS MOLECULAR DEVELOPMENT OF MOTOR NEURONS. Robert G. Kalb and Susan Hockfield, Sect. Neuroanatomy, Yale Univ. Sch. Med., New Haven, CT 06510.

The expression of the cell surface chondroitin sulfate proteoglycan identified by monoclonal antibody Cat-301on motor neurons is dependent on neuronal activity during a circumscribed period in early postnatal life. The NMDA antagonist MK-801 inhibits Cat-301 expression in a stereoselective, dose-dependent manner when administered to neonatal (but not adult) hamsters. We have confirmed that this effect is mediated through the NMDA receptor using a second NMDA receptor antagonist, aminophosphonovaleric acid (APV). APV was incorporated into the polymer Elvax and slices implanted over the lumbar enlargement. In neonates, APV specifically inhibits Cat-301 development on motor neurons in a dose-dependent manner, while in adults APV has no effect on Cat-301 expression.

Two sets of experiments suggest that NMDA receptor antagonists are operating at the spinal segmental level. First, we compared the effects of APV implants placed over the lumbar enlargment to those placed over the cervico-medullary junction (CMJ) or the sensorimotor cortex (SMC). Implants at the lumbar enlargement were twice as potent as CMJ implants in inhibiting Cat-301 expression on motor neurons, while SMC implants had no effect on Cat-301 expression. The decrease in efficacy of APV with increasing distance from the lumbar enlargement suggests that NMDA receptor antagonists exert their effects in this system at the spinal segmental level. Second, to test whether afferents from normally active muscles provide the coherent electrical activity to motor neurons necessary for activation of the NMDA receptor, electrical activity at the spinal segmental level was blocked by implanting a TTX-coated wire into the sciatic nerve. Cat-301 expression was inhibited on motor neurons connected to TTX-silenced muscles. These data suggest that normally active muscles evoke patterned electrical activity at the segmental level, activating NMDA receptors and leading to normal maturation of motor neurons as assayed by Cat-301 expression.

404.3

PHENOTYPIC DIFFERENTIATION OF STRIATAL TRANSPLANTED NEURONS IN RELATION TO DOPAMINERGIC HOST AFFERENTS. B. Onteniente, M. Peschanski and B. Desfontaines*. INSERM U161, 2 Rue d'Alésia, 75014 Paris, France.

The role of dopaminergic (DA) afferents in the acquisition of a specific phenotype by their post-synaptic cells was investigated in embryonic suspensions transplanted into either a kainic acid-lesioned striatum (KA animals) or a KA striatum additionally deprived in DA by 6-OHDA administration (KA-6-OHDA animals). The expression of the D1-associated protein DARPP-32 was observed immunohistochemically at 4 to 60 days following transplantation (T4 to T60) and paralleled with the development of DA afferents from the host. Acetylcholine esterase (AChE) histochemistry was performed on adjacent sections.

Striatal embryonic neurons already expressed DARPP-32 at the time of their dissection (E16). However, no immunoreactivity was observed within the transplants in KA animals until T6. This delay was prolonged until T14 in KA-6-OHDA animals.

As demonstrated by AChE histochemistry, transplants of both experimental groups progressively developed the specific patchy pattern corresponding to the segregation of striatal and extra-striatal tissue. In addition, both the survival and the general morphology of transplanted cells were similar in KA and KA-6-OHDA animals.

Homotopically transplanted striatal neurons, if transiently impaired by their displacement, are able to rapidly recover their specific DARPP-32 phenotype. The role of DA afferents in the acquisition of this expression and in the general development of striatal cells seems to be rather limited since their elimination, although introducing some supplementary delay, does not suppress the expression of DARPP-32.

404.4

DIFFERENTIATION OF ADRENERGIC CELLS IN A CHOLINERGIC GANGLION. R.D.Heathcote, A.Chen and M.Bennett. Department of Biological Sciences, University of Wisconsin, Box 413, Milwaukee, WI, 53201.

While studying the development of a parasympathetic ganglion, we discovered a discrete population of adrenergic cells that differentiated at the same time and in the same location as cholinergic neurons. The cardiac ganglion of the frog, *Xenopus laevis*, contained cells that had the characteristics (small size, catecholamine fluorescence and multipolar cell bodies) of SIF (small intensely fluorescent) cells.

SIF cells were present in some ganglia before cholinergic neurons differentiated. They were also present at 2.1 days of development, when the first cholinergic precursors were becoming postmitotic. Although few SIF cells were in the heart at this stage, they were frequently located close to the heart. SIF cells were associated with differentiated pigment cells that covered the dorsal portion of the yolky endoderm and eventually moved ventrally to cover the peritoneal and pericardial cavities.

There was a slight but steady increase in the average number of SIF cells in the ganglion during early larval development. At 3 weeks, all ganglia contained SIF cells and their average number continued to increase through the end of metamorphosis. Although SIF cells were present before cholinergic neurons, once neurons differentiated (3.3 days), SIF cells were always outnumbered by neurons. Cholinergic neurons became postmitotic and accumulated in the ganglion while SIF cells accumulated at a much slower rate. The accumulation of the two cell types appeared to be coordinated, since SIF cells were maintained at a level one order of magnitude less than cholinergic neurons.

SIF cells were clustered in the sinus venosus portion of the cardiac ganglion. Early in development, the first cholinergic neurons accumulated in the same area. Thus, within this small region, neural crest cells simultaneously adopted two different fates.

404.5

REGULATION OF NEUROTRANSMITTER PHENOTYPE IN THE NEMATODE *C. ELEGANS*. C. M. Loer and C. J. Kenyon*. Dept. of Biochemistry and Biophysics, Box 0554, Univ. of California, San Francisco, CA 94143.

Specification of a neurotransmitter phenotype requires, among other characteristics, the coordinate expression of appropriate biosynthetic enzymes. Synthesis of the biogenic amines dopamine (DA) and serotonin (5HT) require the expression of an aromatic amino acid (AAA) hydroxylase and decarboxylase. In the nematode *C. elegans*, 5HT and DA are used by a number of identified neurons, including a motoneuron required for egg-laying and sensory neurons necessary for male mating. Genes that specify neuronal characteristics in *C. elegans* can be identified by mutation. As a first step towards analyzing the genetic control of neurotransmitter and other neuronal phenotypes, we are cloning genes encoding biosynthetic enzymes for the biogenic amine neurotransmitters to use as markers of specific neuronal differentiation. We used PCR with degenerate oligonucleotides encoding highly conserved regions of AAA hydroxylases to amplify a genomic fragment encoding a *C. elegans* AAA hydroxylase. Of 5 clones sequenced, 2 encoded an AAA hydroxylase; in the region sequenced, 27 of 36 amino acids were identical to human tyrosine hydroxylase. The sequence included a typical *C. elegans* intron at an evolutionarily conserved splice site; typical *C. elegans* codon usage was also apparent in the coding region sequenced. We have isolated a λ genomic clone that we are using to isolate cDNAs for sequencing. We probed a genomic southern blot and found that the gene is present as a single copy in the genome. We plan to use *in situ* hybridization to define sites of expression of the gene. We also plan to fuse β-gal into the AAAH gene and use worms containing such a construct to identify mutations that alter its expression and to examine expression in certain known mutants. Such a screen should yield genes that are required for the regulation of neurotransmitter synthesis as well as genes that determine which neurons produce a specific neurotransmitter.

404.6

COMPARISON OF TYROSINE HYDROXYLASE AND NCAM IMMUNOREACTIVITY IN THE MESENCEPHALON OF THE EMBRYONIC RAT. C.W. Shults and T.A. Kimber*. Neurology Ser., VA Med. Ctr., San Diego, CA 92161 and Dept. of Neurosci., UCSD, La Jolla, CA 92093.

In the developing mesencephalon of the rat, the dopaminergic (DA) cells are generated in the ventricular zone of the basal plate between E11 and E15. The DA cells align and appear to move along radial glia as they migrate from the ventricular zone through the mantle layer to the ventral surface of the mesencephalic flexure. To investigate the specific molecules that control migration of the DA cells, adjacent sections of rat embryos were immunolabelled with a monoclonal antibody that recognizes tyrosine hydroxylase (TH) and a polyclonal antiserum raised against rat NCAM. At E14 and E15, DA cells were noted only along the ventral surface of the mesencephalic flexure. At these ages, NCAM immunoreactive (NCAM-IR) material was noted on the surface of cells in the ventricular zone and throughout the mantle layer. The distribution of NCAM-IR material was not uniform. Cells along the ventral surface of the mesencephalon, the area occupied by DA cells, had the greatest amount of NCAM-IR material. Cells in the ventricular zone contained a moderate amount of NCAM-IR material. Cells between these two regions contained a lower amount of NCAM-IR material. Our data suggest that the level of expression of NCAM on the surface of developing DA cells of the mesencephalon may influence migration and/or expression of TH.

404.7

PRODUCTION AND SECRETION OF NEUROPEPTIDE Y (NPY) BY AGGREGATING FETAL BRAIN CELLS IN CULTURE: A MODEL FOR DEVELOPMENTAL REGULATION OF NPY GENE EXPRESSION. A.Barnea, A.Hajibeigi* and G.Cho*. Depts Obstet. Gynecol. & Physiol., Univ. Texas Southwestern Med. Ctr., Dallas, TX 75235.

NPY is the most abundant peptide in the nervous system. The fetal-rat diencephalon already contains substantial amounts of NPY, which increase dramatically around the time of birth. The aim of this study was to establish an *in vitro* model system for studying the regulation of the expression of NPY gene in the perinatal brain. Single cell suspensions were prepared from the hypothalamic-olfactory tubercle (HT/OT) of 18-day-old rat fetuses, aggregates were formed by constant rotation, and NPY content was assayed by RIA (NPY-IR). Aggregate NPY-IR doubled between 3 to 12 days of culture (1.7 to 3.0 ng/mg P). At each culture-age, the amounts of NPY-IR in the medium exceeded those in the aggregates ≥5-fold. Size-fractionation of NPY-IR indicates the presence of both proNPY-IR and NPY-IR and that their molar ratio is much higher in the medium than in the aggregates. Interestingly, HT/OT of neonates also contained both proNPY-IR and NPY-IR but tissues of 9-day-old rats contained only NPY-IR, suggesting differential regulation of the biosynthesis and processing of proNPY. Thus, the cultured aggregates produce and secrete large amounts of proNPY-IR and NPY-IR, and both production and secretion increase with culture-age. Hence, the cell aggregates provide an excellent model for studying developmental regulation of proNPY biosynthesis/processing.

404.8

Multiple acetylcholine receptor genes are expressed in a tissue-specific fashion in the leech, *Hirudo medicinalis*. Kathleen A. French, Shirley A. Reynolds*, Melissa Hartley*, Steve Heinemann, and William B. Kristan, Jr. Department of Biology, UCSD, La Jolla, CA 92093 and Salk Institute for Biological Research, La Jolla, CA 92037.

Retzius (Rz) neurons in the central nervous system of the leech, *Hirudo*, are alike in all mid-body segments, except in the segments containing the reproductive organs (segments 5 and 6). Although all Rz neurons are indistinguishable from one another early in development, the Rz neurons in the reproductive segments [Rz(5,6)] eventually differ from their segmental homologues in several characteristics, including their physiological response to acetylcholine (ACh) applied to the cell soma. Rz neurons in standard mid-body segments [Rz(X)] depolarize when ACh is applied, but Rz(5,6) hyperpolarize. From experiments with selective blocking agents, we conclude that Rz(X) has one type of ACh receptor (AChRs) in their membranes, while Rx(5,6) must have two types - one which mediates the depolarizing and another the hyperpolarizing responses.

In an attempt to understand the developmental regulation of the two types of AChRs, we have begun to clone AChR genes in *Hirudo*. We used PCR to amplify leech cDNA sequences homologous to published rat and *Drosophila* AChR sequences, and we cloned and sequenced the PCR products. Among the clones are several 300 base-pair oligonucleotides with sequences homologous to AChRs. We hybridized clones to frozen sections of adult leech tissues containing muscle and nerve cells from both reproductive and non-reproductive segments. One clone hybridizes with a specific population of non-neuronal cells in the body wall, and another hybridizes specifically with neurons, but not with muscles. The neuronal AChR clones bind to a sub-set of neurons in all ganglia and hybridize particularly strongly in the neuropil of each ganglion. We conclude that the *Hirudo* genome, like those of other species, includes a family of AChR genes and that these genes are expressed in a tissue-specific fashion in adults. This work was supported by research grants from NIH (NS25916) and the March of Dimes (MOD 1-1064) to WBK and from NIH (NS11549) and the Muscular Dystrophy Association to SH.

404.9

EARLY EXPRESSION OF POTASSIUM CHANNEL TRANSCRIPTS IN THE AMPHIBIAN SPINAL CORD. A. B. Ribera. Dept. of Biol., UCSD, La Jolla, CA 92093 and Dept. of Physio., Univ of CO, Denver, CO 80262.

The delayed rectifier potassium current in amphibian spinal neurons exhibits a specific timetable of functional differentiation, that is coordinated with the maturation of calcium currents to permit developmentally regulated action potential phenotypes. Molecular probes for a *Xenopus* nucleotide sequence encoding a potassium channel have been identified by homology screening with the *Drosophila Shaker* sequence (Tempel et al., 1987). The *Xenopus* sequence is most related to the mammalian sequences RBK2 (McKinnon, 1989) and MK2 (Chandy et al., 1990) and is thus called XSha2. As reported for some mammalian sequences (Chandy et al., 1990;Douglass et al., 1990;Swanson et al., 1990), the coding region is contained within a single uninterrupted exon. Southern analysis of genomic DNA with XSha2 suggests that it is a member of a famiiy of closely related genes. The predicted peptide has 493 amino acids.

Functional expression of XSha2 in oocytes induces a delayed rectifier potassium current. RNase protection assays indicate that XSha2 is expressed in the nervous system but not detectable in the excitable tissues of heart or skeletal muscle. Its transcription is first observed *in vivo* at the neural tube stage and thus slightly after NCAM, an early marker of neural differentiation. In cultured embryonic amphibian neurons, the transcript is present at the time of initial morphological differentiation.

The timing of the functional expression and maturation of the delayed rectifier is fundamental for the characteristic maturation of electrical excitability in amphibian spinal neurons. The tissue-specific and temporal patterns of expression of XSha2 indicate that this program may be established at the level of gene transcription. Supported by NIH grants NS25217 (ABR) and NS25916 to Nicholas C. Spitzer.

404.10

THE MATURATION OF THE DELAYED RECTIFIER POTASSIUM CURRENT IS A CALCIUM-DEPENDENT PROCESS. M.G. Desarmenien and N.C. Spitzer. Department of Biology and Center for Molecular Genetics. UCSD, La Jolla CA 92093.

The delayed rectifier potassium current (I_{Kv}) plays a key role in the differentiation of embryonic amphibian spinal cord neurons: its development converts long lasting calcium-dependent action potentials to fast, primarily sodium-dependent spikes. In *Xenopus* spinal cord neurons maintained in primary culture, the development of I_{Kv} involves a progressive increase in its amplitude during a 24 hr period. In addition, during the last 9 hr, there is a period of maturation during which its rate of rise increases (O'Dowd et al. 1988).

We have examined the influence of calcium influx on the maturation of I_{Kv} by growing mixed neuron-myocyte cultures or neuron-enriched cultures in media deprived of calcium. Removal of calcium from the culture medium demonstrates that the increase in rise time, but not in amplitude, is a calcium-dependent process. Equivalent results are obtained from both enriched and mixed cultures, although the current density is higher in the former condition. The extent of this inhibition depends on the type of culture and the medium used. The sensitivity to this treatment is restricted to the interval between 6 and 15 h in culture. This 9 hr period corresponds to that previously defined as critical for the transcriptional control of the development of I_{Kv} (Ribera and Spitzer,1989). When applied during these 9 hr, 8Br cAMP partially prevents and phorbol ester (PMA) mimicks the effects of low calcium media.

The findings are consistent with the view that the long, calcium dependent action potentials which appear transiently after the neuronal birthdate are a determinant of the maturation of I_{Kv}. The mode of action of calcium is a complex process, in which the activities of protein kinases A and C may be involved.

Supported by the CNRS (MGD) and NS25916 (NCS).

404.11

DIFFERENTIATION OF DELAYED RECTIFIER POTASSIUM CURRENT IN EMBRYONIC AMPHIBIAN MYOCYTES. N.C. Spitzer & A.B. Ribera. Dept. of Biology & Center for Molec. Genetics, UCSD, La Jolla, CA, 92093.

The developmentally regulated expression of prolonged outward potassium currents influences the extent to which sustained inward currents contribute to the action potential at early stages of differentiation. In amphibian spinal neurons, the long duration and calcium-dependence of the embryonic action potential and the amount of calcium influx are largely determined by the extent of maturation of the delayed rectifier potassium current (I_{Kv}; Barish, 1986; O'Dowd et al., 1988; Spitzer, 1988).

We have undertaken a parallel study of differentiation of myocytes, in which action potentials are brief and sodium-dependent even at early stages. The early expression of electrical excitability in embryonic amphibian myocytes growing in culture has been examined previously using intracellular voltage recording techniques (DeCino and Kidokoro, 1985; Henderson and Spitzer, 1986). The membrane exhibits a delayed rectification in response to depolarization at times earlier than those at which impulses can first be generated.

We have examined the differentiation of this outward current in embryonic myocytes developing *in vitro*, using whole cell voltage clamp. I_{Kv} is initially absent. When first recorded it is small and slowly activating but undergoes six-fold increases in both density and rate of activation during the first day in culture. This maturation is dependent upon transcription, and its rate and extent are influenced by the presence of other cell types. The amplitude of the outward delayed rectifier is larger than the inward calcium current at all stages examined and prevents expression of long duration action potentials. Supported by NIH grants NS25916 (NCS) and NS25217 (ABR).

STEROIDS: RECEPTORS AND ACTIONS

405.1

ULTRASTRUCTURAL CHARACTERISTICS OF ESTROGEN RECEPTOR-CONTAINING (ER) NEURONS IN THE VENTROLATERAL NUCLEUS (VL) OF THE FEMALE GUINEA PIG. A.J. Silverman, L. DonCarlos and J.I. Morrell, Dept. Anat. & Cell Bio., Columbia Univ., N.Y., N.Y. and Animal Behavior Inst., Rutgers Univ., Newark, N.J.

Immunocytochemical localization of ER is the only method that allows examination of the ultrastructural characteristics of estrogen receptor cells. We have examined ER neurons in the VL, as a role for these neurons in female sexual behavior has substantial support. Six ovariectomized animals were perfused with 4% paraformaldehyde. ER was localized in vibratome sections using H222, a rat monoclonal anti-ER antibody (Abbott Labs), the Vector Elite kit, and silver intensification of DAB reaction product. Sections were post-fixed in OsO_4 and embedded in EPON. All ER neurons had large cytoplasmic to nuclear ratios; prominent proximal dendrites; a large Golgi apparatus; stacks of rough endoplasmic reticulum and secretory granules. These are characteristics of cells that make peptides. ER neurons showed varying degrees of immunoreactivity that could not be attributed simply to depth within the section. ER was concentrated in the nucleus with the nucleolus being free of deposits. Light reaction product was also seen in both dendritic and somal cytoplasm. The dendrites and cell bodies were heavily innervated with the most common presynaptic element being Gray's Type I terminals (a prominent synaptic cleft, little pre or post-synaptic densities and accumulations of small, round, clear vesicles). The pronounced input to these cells strongly suggests that both synaptic and hormonal signals govern their activity. Supported by HD 10665 (AJS) and HD 22983 (JIM).

405.2

CHANGES IN MBH LHRH AND NPY ASSOCIATED WITH PROGESTERONE AND TRIAMCINOLONE ACETONIDE-INDUCED LH AND FSH RELEASE. D.W. Brann[*1], J.K. McDonald[2], C.D. Putnam[*1] & V.B. Mahesh[*1]. [1]Dept. of Physiol. & Endocrinology, Med. Coll. of GA, Augusta, GA 30912, [2]Dept. of Anat., Emory Univ. Sch. of Med., Atlanta, GA 30322.

In a previous study, we demonstrated that progesterone (P4) and the synthetic glucocorticoid, triamcinolone acetonide (TA), but not cortisol (F), could induce LH and FSH release in estrogen-primed ovariectomized rats. Therefore, the purpose of this study was to determine if the stimulatory effects of P4 and TA on LH and FSH release were associated with changes in LHRH or NPY concentrations in the medial basal hypothalamus (MBH) or preoptic area (POA). Ovariectomized estrogen-primed immature rats received either vehicle, P4, TA or F (1mg/kg BW) at 0900h on day 29. Animals were killed at 0930, 1000, 1200 and 1300h for serum LH and FSH measurements and the MBH and POA were dissected and analyzed for LHRH and NPY concentrations via RIAs. P4 and TA-treated animals showed significantly elevated serum LH and FSH levels at 1300h. F was without effect. P4 significantly increased MBH LHRH and NPY concentrations at 1200h followed by a significant fall at 1300h. TA caused no significant increase in MBH LHRH or NPY at 1200h but, as with P4, there was a significant fall in MBH LHRH and NPY at 1300h. Consistent with its lack of effect on serum LH and FSH, F had no effect on MBH LHRH and NPY. With respect to the POA, P4-treated animals showed a significant fall in LHRH concentrations at 1300h as compared to 1200h. No effect was seen on NPY. TA had no effect on POA LHRH or NPY. Interestingly, F significantly increased POA LHRH and NPY at 1200h followed by a fall at 1300h. However, this change in POA LHRH and NPY may not be related to gonadotropin secretion since F had no effect on LH and FSH levels. In summary, the stimulatory effects of P4 and TA on LH and FSH release appear to be mediated by a similar mechanism involving changes in MBH LHRH and NPY concentrations. Supported by HD16688, HD00727, March of Dimes 1-1118.

405.3

STEROID REMOVAL INCREASES GNRH SECRETION IN THE EWE
SM Moenter, A Caraty*, FJ Karsch* Reprod. Sci. Prog., Dept Physiol., Univ. Michigan, Ann Arbor, MI, INRA Physiol. Reprod., Nouzilly, France.

Changing secretion of gonadal hormones affects the hypothalamo-pituitary axis, altering LH secretion. The extent to which altered LH is due to steroid action on the pituitary or on the brain remains debated. We tested the hypothesis that acute withdrawal of ovarian steroids increases pulsatile GnRH secretion in the ewe. Our approach was to compare GnRH secretion into pituitary portal blood after experimental removal of all ovarian hormones (ovariectomy, OVX) with that in the luteal and follicular phases, i.e. before and after removal of mainly one hormone, progesterone (P). The technique of Caraty and Locatelli (J Reprod Fert 82:263, 1988) was used to collect portal blood samples every 10 min for 6 h from 3 groups of Suffolk ewes in the breeding season: midluteal phase (n=5), early-mid follicular phase (n=5), 24 h after steroid removal (n=10). The latter group was OVX 1 wk before sampling to avoid surgery just before portal blood collection. Estradiol (E) and P were held at luteal phase levels with Silastic implants which were removed 24 h before sampling. Pulses of LH and GnRH were highly coincident. Frequency of GnRH pulses was lower (p=.002) in luteal phase than in OVX ewes (1.2±.4 vs 5.5±.3 pulses/6 hr) and tended (p=.062) to be higher still in follicular phase ewes (8.2±1.3 pulses/6 hr). Amplitude was lower (p=.05) in follicular (1.1±.3 pg/min) than in OVX (6.5±1.7 pg/min) or luteal (4.5±1.8 pg/min) phase ewes (luteal vs OVX NS). Total GnRH detected was higher (p=.01) in OVX (438±98 pg) than in follicular (86±9 pg) or luteal (67±33 pg) phase ewes. We conclude the acute removal of all ovarian hormones causes increased pulsatile GnRH secretion within 24 h. Further, physiologic withdrawal of P at luteolysis, presumably in combination with the rising tide of E, changes the pattern of GnRH secretion such that frequency increases and amplitude decreases in the follicular phase, but may not alter the total amount of GnRH secreted. NIH-18337.

405.4

PROGESTERONE ENHANCES FOS EXPRESSION IN LHRH NEURONS DURING AN LH SURGE.
W.-S. Lee, M.S. Smith, G.E. Hoffman Department of Physiology, University of Pittsburgh, School of Medicine, Pittsburgh, PA 15261.

The ability of progesterone (P) to enhance the LH surge is well established in the rat. However, whether its primary site of action is on the pituitary or brain is unclear. Recently, we have demonstrated that LH surges induced by estradiol benzoate (EB) and P (Hoffman et al., Endocrinology, 126: 1736, 1990) and the events of proestrous (Lee et al., PNAS, 1990, in Press) are accompanied by induction of Fos in LHRH neurons in the preoptic area and anterior hypothalamus, suggesting that stimulated activity of the LHRH neurons is the primary drive for the LH surge. To determine the role of P in this process 1) intact female rats were treated with P antagonist RU 486 (5 mg) at 1230 h on proestrus and killed at specified times during the afternoon and evening for comparison with untreated proestrous rats. 2) Ovariectomized rats were primed with EB (1μg) and then were treated with E alone (50μg) or EB plus P (5mg). The brains were processed for immunocytochemistry of Fos and LHRH. RU 486 treatment dramatically reduced both the magnitude of the LH surge and the degree of Fos induction (numbers of cells expressing Fos and intensity of Fos staining) in LHRH neurons during proestrus. Also, the magnitude of the LH surge and degree of Fos expression in LHRH neurons induced by EB-P treatment were greater than that induced by EB treatment alone. These data suggest that a major site of P action is on hypothalamus and P enhances the LH surge by greatly increasing LHRH neuronal activation. Supported by NIH grant HD 13254 and HD 14643

405.5

EFFECT OF ESTRADIOL (Es) ON NOREPINEPHRINE (NE) LEVELS IN THE PREOPTIC AREA (POA) OF ANESTROUS EWES.
R.L. Goodman, J.E. Robinson*, K.M. Kendrick*, C. Delaney*, and R.G. Dyer*. AFRC Inst of Animal Physiol and Genetics Res, Babraham UK CB2 4AT and Physiol Dept, West Virginia Univ, Morgantown WV 26506

Inhibitory NE input to the POA appears to play a role in the negative feedback action of Es in anestrous ewes. This study examined the effects of Es on NE levels in the POA of ovariectomized (OVX) anestrous ewes and on the response to the α-adrenergic antagonist, phenoxybenzamine (PBZ) using microdialysis probes (5 mm membrane length). Probes were inserted via chronic guide tubes into the POA of conscious ewes and either Ringer's (R) solution (0-8h, 12-20h) or 60 μg/ml PBZ in R (8-12h) pumped through the probes at 2 μl/min. Dialysate samples were collected every 20 min and concentrations of aminergic transmitters and GABA measured using HPLC with electrochemical or fluorescence detectors. Dialysis was done with and without Es (1 cm-long Silastic capsule sc for 2 days) in each of ten ewes. Mean NE levels during 2-8h of dialysis were lower in OVX+Es (477 ± 86 pg/ml) than in OVX (1127 ± 243 pg/ml; $p < .05$), but Es increased the variability of NE (intra-animal CV increased from 16.6 ± 4.3% to 31.6 ± 3.2%; $p < .01$). Es had no effect on epinephrine, dopamine, serotonin, or GABA levels in the POA. Perfusion of PBZ through the probe increased NE levels, presumably by blocking adrenergic autoreceptors. However, this effect only occurred when probes were located rostral to the decussation of the anterior commissure (rostral probes: 424± 60% of controls; caudal probes: 111 ± 18% of controls; $p < .01$), suggesting that the autoregulatory control of NE release varies in different areas in the rostral hypothalamus. In conclusion, these data show that Es decreases mean NE levels in the POA of anestrous ewes, but may increase its episodic release. (HD 17864)

405.6

PROGESTERONE *IN VITRO* RAPIDLY REDUCES NORADRENERGIC AGONIST-STIMULATED cAMP ACCUMULATION IN HYPOTHALAMIC SLICES
N. Petitti and A.M. Etgen, Depts. of Psychiatry and Neuroscience, Albert Einstein College of Medicine, Bronx, NY 10461

We previously demonstrated that norepinephrine (NE) induction of cAMP accumulation in slices of the preoptic area (POA) and middle hypothalamus (MH) is reduced by *in vivo* administration of progesterone (P) to estradiol (E_2)-primed rats Experiments with selective NE ligands indicated that P eliminates α_1 receptor augmentation of β receptor-stimulated cAMP formation (MOLEC BRAIN RES, 5:109-119,1989).

Present studies examined whether *in vitro* exposure to P would also depress NE-stimulated cAMP synthesis. POA and MH slices from E_2-primed females were incubated with 20 nM P for 5-30 min prior to addition of 100 μM NE. Preincubation of slices with P for as little as 5 min significantly suppressed NE-stimulated cAMP formation. This effect was estrogen-dependent in that P did not inhibit NE-stimulated cAMP accumulation in slices from ovariectomized rats. Isoproterenol (β agonist) elevated cAMP to the same extent in slices incubated with and without P; however, phenylephrine (α_1 agonist) was unable to augment cAMP formation in P-exposed slices.

These data indicate that P may have rapid (i.e., non-genomic) effects on α_1-adrenergic receptor coupling to second messenger systems in the hypothalamus.

405.7

DIHYDROTESTOSTERONE INHIBITS ESTROGEN-INDUCED PITUITARY HYPERTROPHY AND HYPERPROLACTINEMIA IN BOTH MALE AND FEMALE RATS.
X.-B. Guan*, A. J. Carrillo, and P. C. Doherty. Dept. of Anatomy, Northeastern Ohio Univ. Col. Med., Rootstown, OH 44272.

Prolonged treatment with estrogen (E) causes pituitary cell hypertrophy, hyperplasia and hypersecretion of prolactin (PRL). We have previously shown that co-administration of dihydrotestosterone (DHT) can inhibit this effect in pituitaries under hypothalamic control, but not those transplanted to an ectopic site. Since the donors used in the transplant study were female rats, the present experiments were undertaken to determine if differences exist between male and female pituitaries in their response to E plus DHT. Adult male and female Fischer 344 rats were castrated and treated with subcutaneous implants of crystalline testosterone (T), E, or E+DHT. Additional groups of males were given ectopic pituitary grafts from male or female donors plus the steroid containing capsules. The E-induced increase in pituitary weight and serum PRL levels was inhibited by co-administration of DHT, but not always to the levels seen in T-treated animals. Though subtle differences existed between male and female rats in the time course of these effects, DHT administration did inhibit the effects of E in both males and females when the pituitaries were under hypothalamic control. These results support our previous contention that DHT can inhibit the effects of E but that hypothalamic input is necessary for these effects to occur. In addition, our results suggest that the primary site of action of DHT to inhibit the effect of E on lactotroph hypertrophy and hypersecretion involves maintenance of the inhibitory activity of tuberoinfundibular dopamine neurons, which is typically altered during prolonged E treatment, or changes in DA receptor sensitivity at the pituitary level. Supported by BMRS Grant No. 2 S07 RR05806-11 and Research Challenge Funds from the Ohio Board of Regents.

405.8

ULTRASTRUCTURAL LOCALIZATION OF ESTROGEN RECEPTOR-IMMUNOREACTIVITY IN GUINEA PIG HYPOTHALAMUS.
J.D. Blaustein, J.C. Turcotte, X. Gu* and M.N. Lehman. Neuroscience and Behavior Program and Psychology Dept., University of Massachusetts, Amherst, MA 01003 and Dept. Anat. and Cell. Biol., Univ. Cincinnati Coll. Med., Cincinnati, OH 45267

We have reported previously that estrogen receptor-immunoreactivity (ER-IR) in the brain is present most abundantly in cell nuclei. However, contrary to most other reports, using enhancement techniques, we have also detected ER-IR in the perikarya and processes of neurons. To determine the organelles with which ER-IR is associated, we developed a technique for ultrastructural analysis of ER-IR. Ovariectomized guinea pigs were perfused, brains vibratome-sectioned, and estrogen receptors immunostained (with H222 antibody) by a multiple-bridge peroxidase-diaminobenzidine technique followed by silver-gold intensification. Electron microscopic analysis revealed distribution of reaction product throughout cell nuclei and in association with a variety of cytoplasmic structures, including free ribosomes, golgi apparatus, lysosomes, and to a lesser extent rough endoplasmic reticulum. Most surprisingly, ER-IR was found associated with small, clear synaptic vesicles (both round and flattened) and synaptic densities associated with some axon terminals. Although further controls are necessary, this suggests immunostaining of the sites of synthesis of the receptor, as well as previously unsuspected sites of action for estradiol.

(Supported by NIH NS 19327, NS 00970 and HD 21968)

405.9

TESTOSTERONE REVERSES AGE-RELATED DECLINE IN VASOPRESSIN GENE EXPRESSION IN THE BED NUCLEUS OF THE STRIA TERMINALIS. D. J. Dobie, M. A. Miller, D.M. Dorsa, GRECC, Seattle Veteran's Affairs Med. Ctr. & Univ.of WA., Seattle, WA 98195. To investigate whether the testosterone (T) sensitivity of arginine vasopressinergic (AVP) cells in the bed nucleus of the stria terminalis (BNST) is preserved in aging, in situ hybridization for AVP was performed in 3 mo. old and 24 mo. old male Fischer 344 rats after castration and replacement for 30 days with either physiologic or supraphysiologic doses of T. Control animals underwent sham surgery. In situ hybridization was performed on 20u sections using a 48-base S ^{35} labelled AVP oligonucleotide probe.

Plasma T levels (ng/ml) were lower in 24 mo. than in 3 mo. old controls (24 mo.: 0.14±.013; 3 mo.: 1.9± 0.661; $p<0.05$), and were higher in supraphysiologically replaced 24 mo. animals than in 3 mo. animals (24 mo.: 7.6±0.371; 3 mo.: 6.5±0.168; $p<0.05$). 24 mo. controls had fewer labelled cells in the BNST than 3 mo. controls (sham 3 mo.: 85±4.1; sham 24 mo.: 30±10.6, $p<0.01$). Supraphysiologic T replacement resulted in an increase in number of labelled cells in both 3 mo. and 24 mo. old animals (sham 3 mo. vs. hi T 3 mo.: 122±7.1; $p<0.05$; sham 24 mo. vs. hi T 24 mo.: 131±9.4; $p<0.001$), with no significant differences in total cell number between the two high T groups. Initial data also revealed an increase in labelling intensity in both groups after T replacement. These results suggest that the decline in AVP gene expression in the BNST of aging rats may be due to the reduction of circulating T in these animals.

405.10

CHANGES IN c-fos EXPRESSION IN THE RAT HIPPOCAMPUS AFTER MANIPULATIONS OF THE BRAIN-PITUITARY-GONADAL AXIS. L. Jennes, Department of Anatomy, Wright State University, School of Medicine, Dayton, OH 45435.

The rat hippocampus has been shown to contain specific receptors for estradiol and gonadotropin releasing hormone (GnRH). In order to determine whether estradiol and GnRH affect transcription in the hippocampal target neurons, the number and distribution of c-fos positive cells was examined with immunohistochemistry after various manipulations of the brain-pituitary-gonadal axis. In the untreated rat, c-fos is expressed by a large number of pyramidal cells throughout CA1 through CA4, certain hilar cells as well as by some granule cells of the dentate gyrus. Ovariectomy results in a significant decrease in the number of c-fos immunoreactive cells throughout all areas of the hippocampus while 1 hour after intravenous injection of estradiol (3 ug/100gBW) the number of c-fos positive cells is restored to control levels. Similarly, intraventricular injections of 1-10 ug GnRH into ovariectomized rats result in the induction of c-fos synthesis in a number of hippocampal neurons which is comparable to control animals. The effects of estradiol and GnRH are strongest between 1 and 4 hours after the treatment and they decline gradually over a period of 24 hours. The results suggest that, in the hippocampus, GnRH and estradiol cause an increase in translation which is at least in part mediated by c-fos. Supported by NIH HD 24697.

405.11

REGULATION OF HIPPOCAMPAL GLUCOCCORTICOID AND MINERALOCCORTICOID RECEPTOR mRNA EXPRESSION BY SEROTONIN. Jonathan Seckl and George Fink Dept. of Medicine, Western General Hospital and MRC Brain Metabolism Unit, Edinburgh, EH4 2XU, UK.

Corticosteroids bind to hippocampal glucoccorticoid (GR) and mineralocorticoid receptors (MR), thereby affecting mood and neurochemical transmission. There is an extensive serotoninergic (5-HT) innervation of the hippocampus which interacts with corticosteroid-sensitive cells. We investigated the effects of 5,7-dihydroxytryptamine (5,7-DHT; 200µg icv) lesions of 5-HT neurons on hippocampal GR and MR mRNA expression in male rats (200g Wistar) by in situ hybridization using specific ^{35}S-labeled cRNA probes. In controls, GR mRNA was highly expressed in dentate gyrus, CA1 and CA2 neurons, but levels in CA3 and CA4 were significantly lower. 5,7-DHT-lesioned animals showed significantly less GR mRNA in dentate gyrus (76% decrease) CA1 (42% decrease) and CA2 (52% decrease). MR mRNA was highly and similarly expressed in all hippocampal subregions in controls. 5,7-DHT led to a significant decrease in MR expression in CA3 (56% fall) and CA4 (45% fall), but not in other subregions. Conversely, treatment of rats with amitriptyline (20mg/kg.dy), which inhibits monoamine re-uptake, led to a significant increase (23±6%) in total hippocampal MR, but not GR mRNA expression (by quantitative slot blotting) after 48 h. After 14 days both MR (87±27%)and GR (56±18%) expression had increased significantly. We conclude that 5-HT may regulate hippocampal corticosteroid receptor mRNA expression in a site-, type- and time-specific manner.

405.12

MINERALOCORTICOID (MR) AND GLUCOCORTICOID (GR) RECEPTORS ARE CO-EXPRESSED IN HIPPOCAMPAL NEURONS M.C.Bohn, E.Howard*, and Z.Krozowski*+. Dept.Neurobiology and Anatomy, Univ. Rochester Med. Ctr., Rochester, NY 14642 and +Prince Henry's Hospital, Melbourne, Australia 3004.

The hippocampus is a major target for glucocorticoid hormones. Previous studies suggest that two receptors are expressed in the hippocampus that bind corticosterone(cort) and are coded for by the MR and GR genes. We have used immunocytochemistry to determine whether neurons in the hippocampus express both receptors. Two polyclonal antisera were used to demonstrate MR-immunoreactivity(IR): MR2, raised against a 16 amino acid synthetic peptide (Endocr.125:192,1989) and MR4,raised against a fusion protein expressed in E. Coli from a construct of glutathione transferase and a 168 amino acid portion of the amino terminus of hMR. A monoclonal antibody against purified liver GR was used to demonstrate GR-IR. Primary hippocampal pyramidal neurons were isolated from E18 rat hippocampus and grown as dissociates.

MR2 and MR4 stained ~100% of pyramidal neurons grown in the presence or absence of serum. In defined medium lacking steroids, MR-IR was observed both in nuclei and cytoplasm and remained in both cellular compartments even after a 30 min. treatment with 10^{-6}M cort. In contrast, GR-IR was barely detectable in serum-free medium, but was present at high levels in cells grown in serum or astrocyte conditioned medium. GR-IR was nuclear in the presence of steroid and cytoplasmic in its absence. Double labeling demonstrated that MR-IR and GR-IR are co-expressed in the majority of pyramidal neurons.

These observations demonstrate that the majority of hippocampal pyramidal neurons co-express MRs and GRs when grown *in vitro* and that different factors regulate expression and cellular localization of the two receptors. Of particular interest is the nuclear localization of MR in the absence of steroid. Since both receptors are expressed in neurons grown in vitro, these systems will be valuable for studying steroid receptor regulation and glucocorticoid regulation of gene expression in the nervous system.

Supported by NIH grant NS20832.

INGESTIVE BEHAVIOR: PEPTIDES II

406.1

RAPID DECREASES IN NEUROPEPTIDE Y (NPY) LEVELS AND RELEASE IN THE PARAVENTRICULAR NUCLEUS (PVN) DURING FEEDING IN RATS ON SCHEDULED FEEDING REGIMEN (SFR). A. Sahu, M.G. Dube, C. P. Phelps and S.P. Kalra, Dept. of Obstet. and Gynecol., University of Florida, Gainesville, FL 32610

Although central administration of NPY readily stimulates feeding in the rat, the pattern of NPY secretion in relation to feeding is not known. We measured NPY concentrations and release from the PVN of adult male rats maintained on 4 h daily food access (1100-1500 h, lights on 0500-1700 h). After 3-4 weeks when body weight and food-intake had stabilized, two experiments were performed. Expt. 1: NPY levels were measured by RIA in 7 microdissected hypothalamic sites of rats on SFR and control rats fed ad libitum. Groups of SFR rats were killed at 1100 h before, and at 1300 and 1500 h after food presentation. Groups of control rats were killed at the same time. In SFR rats, NPY levels in the PVN only were elevated at 1100 h, and steadily decreased to the control range within 2 h after food presentation. Expt. 2: The pattern of PVN NPY release in vivo was correlated with these dynamic changes in PVN NPY concentrations. Push-pull cannulae (PPC) were implanted in the region of the PVN and 1-3 weeks later rats were perfused via the PPC from 1100-1500 h with artificial CSF. Perfusates collected at 10 min intervals were assayed for NPY levels. In control rats, NPY levels in the PVN perfusate were low and remained stable throughout the 4 h-period. However, the secretion of NPY was higher than in controls in SFR rats at the time of food presentation, and thereafter it decreased steadily to reach the control range within 2 h. Collectively, these results show that both NPY levels and release in the PVN are elevated at the onset and decreased during the course of feeding in SFR rats. Consequently, we propose that increased neurosecretory activity in the PVN NPY may play an important role during scheduled feeding (supported by NIH DK 37273).

406.2

FOOD DEPRIVATION INCREASES NEUROPEPTIDE Y (NPY) SECRETION IN THE PARAVENTRICULAR NUCLEUS (PVN): A PHYSIOLOGICAL ROLE FOR NPY IN FEEDING BEHAVIOR IN THE RAT. S.P. Kalra, A. Sahu, M.G. Dube, C. P. Phelps and P.S. Kalra, Dept. of Obstet. and Gynecol., University of Florida, Gainesville, FL 32610

NPY is the most potent orexigenic peptide known. To ascertain whether it is a physiological signal in evoking normal feeding, we studied NPY secretion under conditions that enhance appetite. Our previous studies showed that NPY concentrations changed only in the PVN region in accordance with the change in feeding pattern. Food deprivation (FD) increased and ad libitum feeding returned NPY concentrations to the normal range. We have now assessed NPY secretion in vivo from the PVN of FD and control ad libitum fed rats. Adult male rats were implanted with push-pull cannula (PPC) and 6-8 days later one-half of the rats were FD for three days; water was available ad libitum. On the day of experiment, the PPC assembly was attached to infusion pumps and the PVN was perfused with artificial CSF. After 50 min. of stabilization for baseline, perfusates were collected at 10 min intervals for 60-90 min. Thereafter, FD and control rats received a measured amount of food and sampling continued for an additional 3 h. In the control group, NPY concentrations in the PVN perfusate before and after food presentation, varied between 59-111 pg with an overall mean of 66.7 ± 15.9 pg/10 min. In contrast, NPY concentrations in the FD group were markedly elevated (3-fold) before and after food supply; levels ranged between 121-259 pg with an overall mean of 191.8 ± 23 pg/10 min ($p < 0.01$). The increased PVN NPY secretion in FD rats was associated with 5.3 ± 0.4 g food intake during the collection period. These studies show for the first time that food deprivation increases not only the NPY levels, but also the release of NPY in the PVN. These findings are in accord with our proposal that NPY is a physiological signal responsible for eliciting sustained food intake in FD rats (supported by NIH DK 37273).

406.3

POTENCY OF PEPTIDE YY ANALOGUES FOR STIMULATION OF FEEDING AND IN COMPETITION FOR [^{125}I]-PYY BINDING SITES IN THE HINDBRAIN. E.S. Corp, M. Curcio* and G.P. Smith. Bourne Lab, New York Hospital-Cornell Med. Ctr. White Plains, NY 10605

The relative rank order of potency (ROP) of PYY, NPY and PYY 13-36 to stimulate food intake (90-min test) in nondeprived rats (n=6) following fourth ventricular injection was compared with the ROP of these peptides to compete for saturable [^{125}I]-PYY binding sites in three regions of the hindbrain implicated in ingestion: the area postrema (AP), nucleus tractus solitarius (NTS), and the reticular formation (RF). We report the extrapolated ED_{50} of peptides to stimulate food intake and the IC_{50} values determined from equilibrium-binding experiments coupled with quantitative receptor autoradiography.

	FEEDING ED_{50} (pmole)	BINDING IC_{50} (nM)		
		AP	NTS	RF
PYY	50	0.7	2.2	0.2
NPY	100	1.2	2.5	3.1
PYY 13-36	>2400	31.1	37.0	7.2

The ROP of peptides to stimulate food intake most resembles their ROP to compete for [^{125}I]-PYY binding in the AP. This suggests the AP as a target for the action of PYY and NPY in feeding. Moreover, the lack of potency of the selective Y_2 agonist PYY 13-36 suggests that the feeding response is mediated by a different PYY-receptor subtype (Y_1, most likely). The greater competitive potency of PYY 13-36 in the RF, compared with its competitive potency in the AP and NTS, suggests that the RF is a region enriched with Y_2 receptors.

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406.4

INTRAPORTAL PANCREATIC GLUCAGON INFUSIONS FAIL TO REDUCE SPONTANEOUS MEAL SIZE IN HEPATIC VAGOTOMIZED RATS. N. Geary, J. Le Sauter, U. Noh*. Psychology Dept., Columbia Univ., NY, NY 10027.

Remotely controlled, meal-contingent intraportal infusions of pancreatic glucagon reduce the size of rats' spontaneous meals both early and late in the dark phase. Glucagon administration also inhibits elicited feeding during test meals, and under some conditions, this is blocked by selective vagotomy of the hepatic branch of the vagus. We investigated whether glucagon's effect on spontaneous feeding is also dependent on the hepatic vagus. Hepatic-vagotomized and neurologically intact rats were intraportally infused with 13.6 µg (.034 ml/min x 2 min) glucagon or vehicle as they began the first spontaneous meal 9 h after dark onset. Glucagon failed to inhibit feeding after hepatic vagotomy:

	Meal Size (g, m + se)	
	Intact (n=15)	Vagotomized (n=13)
Vehicle	3.6 + 0.3	3.3 + 0.4
Glucagon	2.4 + 0.2*	3.0 + 0.2

*p < .01 vs. vehicle, ANOVA & Bonferroni test

We conclude that the hepatic branch of the subdiaphragmatic vagus is necessary for the satiety effect of exogenous glucagon on spontaneous feeding late in the dark phase.

406.5

BLOCKADE OF THE CENTRAL BOMBESIN (BN) RECEPTORS ENHANCES FOOD INTAKE IN PREFED RATS.

Z. Merali[1], T.W. Moody[2] and D.H. Coy[3]. [1]Psychology & Pharmacology, Univ. of Ottawa, Ontario, K1N 9A9. [2]Biochemistry, George Washington Univ. Washington D.C. 20037. [3]Dept. of Medicine, Tulane Univ. New Orleans, LA 70112.

BN-like peptides have been found to induce satiety-like state in several species including rat, fowl, mouse, pig, baboon and man. A definitive test of the involvement of endogenous BN-like peptides in feeding behavior has been hindered by a lack of potent and selective BN antagonist(s). We have previously shown that ΨLeu^{13,14}-BN can block suppression of food intake induced by exogenous BN. In second messenger assays, ΨLeu^{13,14}-BN also antagonized the ability of BN to elevate cytosolic Ca^{++}. The current study attempted to determine if and under what conditions this antagonist would antagonize/postpone satiety. Male Sprague-Dawley rats (350 to 400g), were food deprived for 17 hr, administered the antagonist (icv) and then given access to food (standard rat chow pellets) for 60 min 'test meal'. In this paradigm blockade of the central BN receptors (with 0.5 to 20 ug ΨLeu^{13,14}-BN, icv) did not significantly enhance the size of the test meal. In the next set of experiments, the rats were first allowed to prefeed (for 45 min) and then administered ΨLeu^{13,14}-BN, followed by presentation of the 60 min 'test meal'. In this case, blockade of the central BN receptors significantly enhanced the consumption of the 'test meal'. This increment in food intake was dose-dependent, starting at the 10 ug dose and reaching maximal effect at the 20 ug dose. In vitro autoradiographic studies revealed that ^{125}I-Tyr^4-BN bound with IC_{50} value of 100 and this binding was greatly reduced in the presence of 1 uM ΨLeu^{13,14}-BN, particularly at various hypothalamic nuclei. This, to our knowledge, is the first report to illustrate enhancement of food intake following blockade of the central BN receptors. The data support the contention that endogenous BN-like peptides may play a role in regulation of food intake. (Supported by MRC).

406.6

THE SATIATING EFFECT OF BOMBESIN IS MEDIATED BY RECEPTORS PERFUSED BY THE COELIAC ARTERY. T.C. Kirkham, J. Gibbs and G.P. Smith. The NY Hospital-Cornell Medical Center, White Plains, NY 10605 USA

We have suggested that the satiety action of exogenous, peripherally administered bombesin (BBS) may be mediated by the stomach (Gibbs and Smith, 1982). To assess this possibility, male Sprague Dawley rats were surgically prepared with chronic arterial catheters. Catheter tips were sited within either the coeliac a. (n=9) or superior mesenteric a. (n=6). Two min prior to 60-min feeding tests (liquid diet: 40% v/v BioServ), nondeprived rats received bolus infusions (1 ml kg^{-1} over 10s) of 0.15M NaCl or BBS (Bachem; 1, 2 or 4 µg kg^{-1}). A separate group of unoperated rats (n=6) was tested after i.p. injection of the same doses.

Maximum suppression of intake was observed after 15 min of testing. Examination of the data revealed that over this interval, BBS delivered into the coeliac a. had markedly greater satiating potency than via other peripheral routes (order of potency: coeliac > sup. mesenteric a. = i.p.). The table shows mean (±SEM) per cent reduction at 15 min produced by BBS by each route (* p<.05, ** p<.01: significantly different from coeliac value at the same dose).

	Dose (µg kg^{-1})		
Route	1	2	4
coeliac a.	35.3 (6.1)	55.3 (8.8)	61.5 (7.9)
sup. mesenteric a.	8.6 (9.1)**	27.8 (12.5)**	22.9 (11.8)**
intraperitoneal	20.4 (11.3)	20.3 (5.7)**	35.3 (8.1)**

Since equivalent doses of BBS produce significantly more inhibition of food intake when administered into the coeliac a. than into the superior mesenteric a., we conclude that receptors mediating the satiating effect of BBS are concentrated in the abdominal organs perfused by the coeliac artery. These organs include the stomach, proximal duodenum, pancreas and liver.

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406.7

CCK-A BUT NOT CCK-B ANTAGONIST ATTENUATES SUPPRESSION OF FOOD INTAKE BY EXOGENOUS CCK OR INTRAINTESTINAL OLEATE. L.A. Brenner and R.C. Ritter. Dept. of VCAPP, Washington State University, Pullman, WA 99164-6520.

We have reported that suppression of sham feeding by intraintestinal oleate is attenuated by the CCK type A (CCK-A) receptor antagonist, MK-329, suggesting that endogenous CCK, acting at type A receptors, participates in suppression of feeding by oleate. Recent reports suggest, however, that CCK type B (CCK-B) receptors also participate in control of food intake. Therefore, we are comparing the efficacy of the CCK-A receptor antagonist (MK-329) with that of the CCK-B antagonist (L-365,260) for their ability to attenuate suppression of feeding by exogenous CCK or intraintestinal oleate infusion. MK-329 at a dose of 0.37 µmol/kg abolished suppression of real feeding by intraperitoneal CCK-8 (2 µg/kg). An equivalent dose of L-365,260 had no effect on suppression of feeding by CCK-8. In confirmation of our previous finding, 1.47 µmol/kg MK-329 abolished suppression of sham feeding by intraintestinal oleate. L-365,260 at doses of 0.37, 0.74 or 1.47 µmol/kg failed to attenuate suppression of sham feeding by oleate. These data support the conclusion that systemic injection of exogenous CCK suppresses feeding via CCK-A receptors. Likewise, our results suggest that suppression of sham feeding by intraintestinal oleate is mediated by endogenous CCK, acting at type A receptors. Supported by NIH grant NS20561.

406.8

COMPARATIVE EFFECTS OF CHOLECYSTOKININ (CCK-A) RECEPTOR ANTAGONIST L 364718 AND CCK-B RECEPTOR ANTAGONIST L 365260 ON SOLID FOOD INTAKE IN RATS. RD Reidelberger, G Varga*, TE Solomon*. Dept Physiol and Med, Univ Kansas Med Sch, Kansas City, KS 66103; VA Med Ctr, Kansas City, MO 64128.

Cholecystokinin's (CCK) role as an important physiological satiety factor has been supported recently by studies showing that the selective CCK-A receptor antagonist L 364718 stimulates food intake in several species. The inhibitory effect of CCK on feeding has generally been thought to be mediated by CCK-A rather than CCK-B receptors, because L 364718 blocks suppression of feeding by exogenous CCK-8, and because the selective CCK-B agonist desulfated CCK-8 has no effect on food intake when administered centrally or peripherally. It was recently reported, however, that the selective CCK-B antagonist L 365260 is 100 times more potent than L 364718 for stimulating feeding and preventing satiety in partially satiated rats (C. T. Dourish et al., *Science* 1989). Thus, the role of CCK-B receptors in control of food intake is not clear. In the present study we compared the dose-response effects of L 364718 and L 365260 on food intake during the dark period in ad libitum feeding rats. Male, Sprague Dawley rats (300–400 g) were adapted to a reversed light cycle (lights off from 1000–2200 h) and provided excess ground rat chow daily at the beginning of the dark period. Rats received L 364718 (0, 1, 10, 100, 1000 µg/kg ip) or L 365260 (0, 0.1, 1, 10, 100, 1000, 10,000 µg/kg ip) 2 h after lights off. Food intake was measured 1.5, 3.5, and 5.5 h later. L 364718 significantly stimulated 1.5-h food intake by ~40% at 10 µg/kg and higher doses; cumulative intake at 3.5 and 5.5 h remained elevated by ~20% at 1000 and 100 µg/kg, respectively. In contrast, L 365260 had no significant stimulatory effect on feeding at any dose. ***Conclusion***: These results suggest that CCK interacts with CCK-A receptors to produce satiety during the dark period in ad libitum feeding rats.

406.9

TYPE A AND B CHOLECYSTOKININ (CCK) RECEPTORS IN THE INHIBITION OF FOOD INTAKE PRODUCED BY EXOGENOUS AND ENDOGENOUS CCK. T.H. Moran, P.J. Ameglio* and P.R. McHugh. Department of Psychiatry, Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205.

Recent work has suggested a role for an endogenous central release of CCK acting at type B CCK receptors in the control of food intake. In an effort to investigate whether the mechanism by which peripheral CCK inhibits food intake is through the stimulation of a central CCK release, we examined the ability of the A (MK-329) and B (L-365,260) CCK antagonists to block the inhibition of glucose consumption in a 1 hr test produced by an IP injection of 4 ug/kg of CCK in 6 hr deprived rats. At 15 minutes, increasing dosages (10 - 100 ug/kg) of the type A antagonist resulted in a blockade of the inhibition of intake produced by CCK. At 60 minutes, when the effect of exogenous CCK was no longer present, 100 ug/kg of the A antagonist significantly increased glucose consumption above baseline levels. Thus, not only were the effects of exogenous CCK blocked by the A antagonist, but the effects of endogenous CCK were antagonized as well (with this deprivation, rats enter the test with chow in their stomachs). In contrast, no dose (10 - 1000 ug/kg) of the B antagonist blocked the inhibitory action of the exogenous CCK at 15 or 30 min nor resulted in increased intake at 60 min. These results demonstrate that the effects of exogenous CCK are not mediated through type B CCK receptors and that the A antagonist can stimulate intake in situations in which the B antagonist cannot. (Supported by DK19302)

406.10

EFFECT OF CHOLECYSTOKININ (CCK-8) ON GASTRIC PRESSURE AND REPORT OF GASTRIC FULLNESS IN WOMEN. P.M. Melton, H.R. Kissileff, and F.X. Pi-Sunyer*. Obesity Research Center, St. Luke's-Roosevelt Hospital & Columbia University.

CCK-8 may affect food intake by augmenting neural activity from the distended stomach, thereby amplifying satiety signals (Muurahainen, N. et al., Physiol. & Behav., 44: 645-649, 1988). In order to test the hypothesis that gastric pressure changes are involved in amplifying satiety signals, a gastric balloon was inflated in the stomach of each of four women with CCK-8 (112 ng/min) or saline infusion. Balloons were inflated to 500 ml or to the maximum volume tolerated by the subject. When the balloon was inflated to 500 ml, there were no differences in gastric pressure between the CCK-8 and saline conditions. Nonetheless, ratings of fullness were higher with CCK-8 administration (5.75) than with saline administration (4.50). Unexpectedly, the volume tolerated with CCK-8 infusion (859 ml) was slightly larger than with saline infusion (695 ml). The pressure rise, however, was significantly smaller with CCK-8 (8.0 cm) than with saline (11.8 cm). The slope of the relation between fullness ratings and gastric pressure was significantly steeper when CCK-8 was infused. In addition, gastric contractions were practically abolished with CCK-8 infusion. CCK-8 appears to relax the stomach and at the same time sensitize it to gastric pressure.

406.11

SPECIFIC INSULIN ANTIBODIES INCREASE FOOD INTAKE IN RATS WHEN CHRONICALLY INFUSED INTO THE VENTROMEDIAL HYPOTHALAMUS. M.K. McGowan, K.M. Andrews* and S.P. Grossman. Committee on Biopsychology, University of Chicago, Chicago, IL 60637.

We (McGowan, M.K., et al., Behav. Neurosci. 104:371, 1990) have shown that chronic intrahypothalamic (IH) infusion of insulin results in dose-dependent decreases in body weight and food intake. In order to determine whether exogenous insulin is acting on an endogenous insulin-receptive system, we infused specific insulin antibodies (IRI Ab) or 0.9% saline into the ventromedial hypothalamus in rats. Experimental animals received IH saline for 1 wk followed by IRI Ab (3.0 μUeq/hr) for 1 wk. Control animals received IH saline for 2 weeks. Experimental animals' daily food intake (FI) during saline infusion was not significantly different from controls'. Experimental animals' daily FI increased during IRI Ab infusion compared to their FI under saline infusion (from 25.4±1.35 g to 29.3±1.14 g, $t=2.23$, $p<0.05$) and to controls' (25.0±1.04 g, $t=2.78$, $p<0.01$). In the wk which followed IRI Ab infusion, experimental animals' FI decreased to 27.8± 1.65 g/day, a level which is not significantly different either from their FI during saline infusion or from controls' (25.6±0.80 g/day). These data provide further evidence that the hypothalamus contains an insulin-sensitive system which is involved in food intake regulation.

406.12

SATURABLE TRANSPORT OF PLASMA INSULIN INTO THE CENTRAL NERVOUS SYSTEM (CNS). M.W. Schwartz, S.E. Kahn*, G.J. Taborsky Jr.*, R.N. Bergman*†, and D. Porte, Jr.* Dept. of Medicine, Univ. of Washington, Seattle WA 98195 and †Dept of Physiology and Biophysics, U.S.C., Los Angeles, CA 90024.

The ability of plasma insulin (I) to enter the brain and to modify food intake has been implicated in body weight regulation. By mathematical modeling of kinetic data, we have recently shown that plasma I enters cerebrospinal fluid (CSF) only after traversing an intermediate compartment, which we hypothesize to be brain interstitial fluid (ISF) (Clin Res. 38:125A, 1989). Thus, the primary route of I entry into CSF may be across the blood-brain barrier (BBB) endothelium, rather than the blood-CSF barrier. To test the hypothesis that this uptake occurs via a saturable transport process, we studied anesthetized dogs (n=16) maintained euglycemic during i.v. infusion of I for 2.5 hrs to achieve stable plasma levels of I ranging from 80-3500 μU/ml. The kinetics of uptake of I from plasma into CSF over 7.5 hours were analyzed with a model that permitted calculation of the rate (V) at which plasma I enters the intermediate compartment (brain ISF) (data as $\bar{x} \pm$ SEM):

Plasma I (μU/ml)	122±2	207±6	705±56	2553±274
V (μU/min)	18.1±4.3	20.5±6	67±5	134±33

These data demonstrate Michaelis-Menten type (i.e., saturable) kinetics; i.e., a 20-fold increase in the plasma level produced only a 7.5-fold rise in the transport rate. Lineweaver-Burk analysis revealed a V_{max} of 75 μU/min and K_M of 3-4 nM, similar to the K_D of the I receptor.

We conclude that plasma I is transported into the CNS via a saturable process. I receptors located on the BBB endothelium are likely to mediate this transport.

LONG-TERM POTENTIATION V

407.1

INCREASE IN THE PROPORTION OF PERFORATED AXOSPINOUS SYNAPSES INDUCED BY LONG-TERM POTENTIATION (LTP). Y. Geinisman, L. deToledo-Morrell and F. Morrell. Dept. of CMS Biol., Northwestern Univ. Med. Sch. and Depts. of Neurol. Sci. and Psychol., Rush Med. Coll., Chicago, IL 60611.

Previous work using biased methods for synapse quantitation failed to consistently demonstrate an LTP-induced increase in synaptic numbers which could underlie a sustained augmentation of synaptic efficacy during LTP. This study was designed to reexamine the issue with the aid of an unbiased technique. Young adult rats were implanted with stimulating electrodes in the medial perforant path and recording electrodes in the hilus of the ipsilateral dentate gyrus. Potentiated animals (n = 7) were stimulated (with fifteen 20 ms bursts of 400 Hz delivered at 0.2 Hz) on each of 4 consecutive days and sacrificed 1 hr after the fourth stimulation. Unpotentiated but stimulated (coulombic controls) and unstimulated but implanted rats served as controls (n = 7 in each). The number of synapses per neuron was estimated in the middle molecular layer of the dentate gyrus using the disector technique. Results showed that the induction of LTP was not accompanied by any increase in the total number of synapses on dendritic shafts or spines. Further subdivision of axospinous synapses into perforated (with a discontinuous postsynaptic density, PSD) and nonperforated (with a continuous PSD) ones revealed a marked (24 and 38%) and statistically significant ($p < 0.02$) increase in the proportion of perforated to nonperforated synapses in potentiated animals compared to unstimulated and coulombic controls, respectively. This alteration is similar to, though less prominent than, the synaptic restructuring described for rats kindled through the same pathway (Geinisman et al., *Brain Res.*, 1990, *507:* 325). It suggests that the structural synaptic modifications underlying LTP may be viewed as an initial stage of those that ultimately lead to kindling.

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407.2

SELECTIVE REDUCTION IN THE AVERAGE SIZE OF PERFORATED POSTSYNAPTIC DENSITIES IN LONG-TERM POTENTIATION (LTP). L. deToledo-Morrell, Y. Geinisman and F. Morrell. Depts. of Neurol. Sci. and Psychol., Rush Med. Coll. and Dept. of CMS Biol., Northwestern Univ. Med. Sch., Chicago, IL 60612.

Sustained enhancement of synaptic efficacy characteristic of LTP could be associated with an enlargement of the postsynaptic density (PSD) which is the site of greatest concentration of postsynaptic neurotransmitter receptors and ion channels. Previous studies provided conflicting results regarding the effect of LTP on PSD dimensions. Earlier work was based on the use of random sections in which synapses cannot be accurately identified and sampled. To circumvent these problems, we undertook a serial section study. Young adult rats were implanted with stimulating electrodes in the medial perforant path and recording ones in the hilus of the ipsilateral dentate gyrus. Potentiated animals (n = 7) were stimulated (with fifteen 20 ms bursts of 400 Hz delivered at 0.2 Hz) on each of 4 consecutive days and sacrificed 1 hr after the fourth stimulation. Unpotentiated but stimulated (coulombic controls) and unstimulated but implanted rats served as controls (n = 7 in each). Unbiased PSD samples were obtained from the middle molecular layer of the dentate gyrus using the disector technique. For each PSD sampled, all profiles were measured in serial sections to assess the PSD maximal profile length and the area of the PSD plate (Brændgaard & Gundersen, *J. Neurosci. Meth.*, 1986, *18:* 39). The only significant change found in potentiated rats was a selective diminution of PSD dimensions in perforated axospinous synapses with discontinuous PSDs (but not in nonperforated ones with continuous PSDs or in synapses on dendritic shafts). We propose that the process of synaptic remodelling in which nonperforated synapses with relatively smaller PSDs are converted into perforated ones may be intensified by LTP. Thus, the reduction in the mean size of perforated PSDs may reflect an increase in the number of newly formed and immature perforated synapses.

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407.3

EFFECT OF URETHANE ANESTHESIA ON LONG-TERM POTENTIATION, PARTIAL KINDLING, AND KINDLING-INDUCED POTENTIATION IN THE PERFORANT PATH-DENTATE GYRUS CIRCUIT OF THE RAT. D. P. Cain, F. Boon* and E. L. Hargreaves. Dept. of Psychology, Univ. of Western Ontario, London, N6A 5C2, CANADA.

Urethane anesthesia is commonly used in studies of LTP, but it blocks amygdala kindling (Life Sci, 1989, 44:1201). In order to study the relation between LTP, kindling and kindling-induced potentiation we administered urethane (1.5 or .75 g/kg) to rats that carried perforant path (PP) stimulating and dentate hilus recording electrodes. Urethane had a strong attenuating effect on LTP of the pop spike ($p<.005$), reducing LTP to <30% of that of saline controls, although there was significant LTP under urethane. Urethane had a dose-related suppression effect on growth of 4 ADs subsequently elicited at hourly intervals in the same rats by stimulation of the PP. The 1.5 g/kg rats failed to exhibit any partial kindling. Partial kindling failed to augment the pop spike and had a significant suppressant effect on the amplitude of the previously potentiated pop spike in the control rats. These data suggest a dissociation between the effect of urethane on LTP and partial kindling in the same brain structure. Supported by NSERC/Canada.

407.4

ANOXIA DOES NOT ABOLISH LTP. Y. Xu and K. Krnjević. Anaesthesia Research Dept., McGill University, Montréal, P.Q., Canada H3G 1Y6.

Anoxia grossly disrupts cognitive function, and can produce retroactive amnesia. It was therefore of interest to see how anoxia affects long-term potentiation (LTP) in hippocampal slices. In experiments on slices from Sprague-Dawley rats, EPSP fields were evoked in areas CA1, CA3 and the dentate gyrus by stimulating the appropriate afferent pathways. LTP was elicited by two brief tetani (100 Hz for 1 s, separated by 20s). It was manifested by a near
50% enhancement of the EPSP field, which persisted during further periods of recording of at least 30-60 min. Anoxia (N_2 was substituted for O_2 in the aerating gas) typically lasted 3 min, long enough for nearly complete (>90%), but reversible, suppression of synaptic responses. When tetanic stimulation was applied during anoxia, LTP consistently failed to develop. But if anoxia was applied 5-30 min after the end of tetanic stimulation - when LTP had appeared - the temporary suppression of EPSPs was followed by a return to the potentiated level. Findings were similar in all three regions, CA1, CA3 and the dentate gyrus.

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407.5

FREE RADICALS ACCELERATE THE DECAY OF LONG-TERM POTENTIATION (LTP). G. E. Hollinden, J. M. Sarvey, and T. C. Pellmar, Physiology Dept., AFRRI and Dept. of Pharmacology, USUHS, Bethesda, MD 20814.

Previous studies have shown that H_2O_2 ($\geq$ 0.005%) generates free radicals in slices of hippocampus and impairs synaptic transmission and spike generation. In the present study we evaluated the sensitivity of long term potentiation to free radical damage.

A bipolar stimulating electrode was positioned in s. radiatum of guinea pig hippocampal slices. Population spikes (PS) and synaptic potentials (pPSP) were recorded from s. pyramidale and s. radiatum of field CA1. LTP was produced by high frequency stimulation (HFS; 100 Hz, 1 sec) at a stimulus strength that produced a half-maximal PS. Tissue was treated with a concentration of H_2O_2 (0.002%) that had no apparent effects on evoked responses.

In untreated slices (n=17) LTP was sustained for at least 60 min but in slices treated with H_2O_2 (n=16) potentiation of both PS and pPSP decayed. H_2O_2 treatment beginning 5 min after HFS had no effect on LTP (n=9). Washout of H_2O_2 following HFS allowed some but not total expression of LTP (n=16). Short-term potentiation ($\leq$15 min) was unaffected by H_2O_2 exposure.

These data suggest that free radicals prevent maintenance of potentiation by interfering with a process during the induction phase of LTP.

407.6

EFFECTS OF CHRONIC ETHANOL EXPOSURE ON LONG TERM POTENTIATION IN THE CA1 REGION OF THE HIPPOCAMPUS. M.F. Tremwel and B.E. Hunter, Dept. of Neuroscience, University of Florida, Gainesville, FL 32610.

Chronic ethanol treatment (CET) results in a depressed ability to induce long term potentiation (LTP) of the population spike (PS) in the CA1 region of the hippocampus. This study examined the effects of CET on the synaptic component (population excitatory post-synaptic potential (EPSP)) of LTP. We compared the relative contribution of EPSP versus PS potentiation after CET. Ethanol-treated (E) and control (S) groups were fed a liquid diet containing either ethanol or sucrose for 28 weeks followed by an 8 week abstinence period. Hippocampal slices were prepared and extracellular field recordings obtained from area CA1 of the hippocampus. A stimulating electrode was placed in stratum radiatum (SR) and parallel recording microelectrodes were placed in SR and stratum pyramidal (SP). To study the characteristics of EPSP and PS LTP induction, we applied a series of high frequency stimulus trains of increasing duration. Stimulus strength was maintained at PS threshold. Following each conditioning train, recordings were obtained from SR and SP until EPSP and PS values returned to baseline. LTP was assessed by comparing the EPSP slope and PS amplitude after the conditioning train with the baseline values obtained in the absence of high frequency stimulation.

CET altered the relationship between EPSP and PS potentiation during induction of LTP. These results suggest that the mechanisms involved in the induction are susceptible to the toxic effects of CET. Current research is aimed at examining specific components of the induction process to determine the mechanism by which CET alters LTP.

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407.7

ADRENAL STEROIDS, LONG-TERM POTENTIATION AND DEPRESSION. D. Filipini*, K. Gijsbers*, M.K. Birmingham* and B. Dubrovsky. Dep. of Psychiatry, McGill Univ. Montréal QC H3A 1A1.

Previous work (Dubrovsky et al "Steroids and Neural Activity" CIBA Symposium 153) revealed that adrenal steroids and their ring-A reduced metabolites can affect short as well as long-term, such as LTP, neuronal activity. The present report extends these results showing that LTP recorded from the granular cell layer of the dentate gyrus of ADX rats after tetanic stimulation of the perphorant path, was affected as follows:

18-OH-deoxycorticosterone, produced a marked decrease of the EPSP's values and arrested the PS LTP at every time post-tetanic stimulation (PT) compared to the injection of vehicle (V), Nutralipid 10%. Its 21-acetate moderately decreased the EPSP and had no effect on the PS. Deoxycorticosterone (DOC) also decreased all EPSP's values while the PS was reduced in the first 30' PT. Corticosterone (B), decreased both the EPSP and PS for the first 15' and 30' PT respectively. B-acetate, showed initial decrease of the EPSP and no effect on the PS. Allotetrahydro-DOC showed almost no effect on LTP but an inital enhancement of the PS. Allotetrahydro-progesterone, decreased all EPSP's values and had no effect in the PS development in comparison with V.

As therapeutic implication of this work, steroid modulation of a putative memory mechanism, we have treated depressed patients with adrenal gland hyperactivity and memory biasing by adrenal supression with ketoconazole, a steroid synthesis inhibitor. Preliminary results revealed a fast relief of the severe mood disturbances in five (5) patients.

407.8

MU BUT NOT DELTA OPIOID RECEPTOR ANTAGONISTS BLOCK THE INDUCTION OF LONG-TERM POTENTIATION AT THE MOSSY-FIBER CA3 SYNAPSE OF THE RAT HIPPOCAMPUS IN VIVO. D.N. Lieberman, B.E. Derrick, and J.L. Martinez, Jr. Department of Psychology, Univ. of California, Berkeley, CA 94720

Previously we reported that the opioid receptor antagonist naloxone prevents induction of mossy fiber (MF)-evoked, but not commissurally (COM)-evoked LTP recorded in the CA3 region, without affecting baseline responses in either pathway. In this study, we found that local application to the CA3 pyramidal layer of $Cys^2, Tyr^3, Orn^5, Pen^5$amide (CTOP), an antagonist selective for mu opioid receptors, did not affect baseline field EPSP responses evoked extracellularly in either the MF or COM afferents in pentobarbital anesthetized animals. One µmol CTOP prevents induction of MF-, but not COM-, evoked LTP following two 100 Hz 1 sec conditioning trains. Naltrindole hydrochloride (NTI), an antagonist selective for delta opioid receptors, in quantities of 1 and 10 µmols did not impair induction of LTP in either the MF or COM pathway. These data suggest that mu, but not delta, opioid receptor activation may be required for induction of LTP at the MF-CA3 synapse in vivo. Norbinaltorphimine, an antagonist selective for kappa opioid receptors, at quantities equimolar to CTOP and NTI, affected baseline responses in both pathways, and therefore a role for kappa receptors in induction of MF-CA3 LTP cannot be excluded. Supported by DA04195, DA05374, and the Rennie Fund.

407.9

AGE-DEPENDENT EFFECTS OF SEX STEROID HORMONES ON HIPPOCAMPAL LTP IN VITRO. K.L. SKINKLE*, K-I. ITO*, W.L. SALINGER AND T.P. HICKS, Dept. of Psychology, UNC-Greensboro, Greensboro, NC, 27412-5001.

Wistar rats of both sexes, ranging in age from 18 days to adulthood were decapitated and the dissected hippocampi were sliced and maintained in vitro following standard procedures. Long-term potentiation (LTP) of CA1 responses was induced following brief tetanic stimulation (100 Hz, 1 s) of the Schaffer collateral-commissural pathway. There was no suppression evident of the magnitude of the LTP of evoked CA1 population spikes in adult (3 mo.) males or females using a concentration of 10^{-8} M 17-ß estradiol (ß-E). By contrast, in 4-wk-old animals of both sexes, ß-E (10^{-8}M) potently, rapidly, reversibly, and dose-dependently suppressed LTP. There was no observed suppressant effect of progesterone (10^{-8}M) or testosterone (10^{-8}M) on LTP from animals of this age. 3-wk-old hippocampi from females exhibited similar suppressant effects of ß-E, but the steroid appeared significantly less potent on these younger animals. Tamoxifen (10^{-6} M), an anti-estrogen compound that has effects upon intracellular ß-E recognition sites, was without effect in diminishing the suppressant effect of ß-E (10^{-8}M) on 4-wk-old females. These data suggest a possible age-dependent role of ß-E in modulating the efficacy of synaptic mechanisms underlying hippocampal LTP during a critical developmental period.

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407.10

THE NON-NMDA COMPONENT OF LTP IN THE CA1 AREA OF RAT HIPPOCAMPAL SLICES. K-I. Ito*, K.L. Skinkle*, K. Takeda* and T.P. Hicks, Dept. of Psychology, UNC-Greensboro, NC, 27412.

Ever since the observations of Collingridge et al. (1983), it has been recognized that long-term potentiation (LTP) requires the activation of a subtype of receptors for excitatory amino acids (EAAs), the N-methyl-D-aspartate (NMDA) receptors. Antagonists that are selective pharmacologically for these NMDA receptors comprise 2 major classes: ω-phosphonic α-carboxylic acids (eg: AP5; D-2-amino-5-phosphopentanoate) and D-monoamino dicarboxylic acids (eg: DαAA, D-α-aminoadipate). LTP incluced by brief, high-frequency stimulation (HFS, 100 Hz, 1 s) of the Schaffer collateral-commissural pathway of hippocampal slices from rats was suppressed partially by DαAA at relatively low (200 μM) as well as at relatively very high (1 mM) doses. By contrast, as has been reported by Collingridge et al. (1983), the relatively more potent NMDA-selective antagonist, AP5 (30-50 μM), suppressed LTP completely. To test if the incomplete suppression of LTP by DαAA was due simply to its lower potency at NMDA receptors, intracellular recording was used to contrast these doses of DαAA and AP5 on NMDA-elicited responses. Both AP5 (30-50 μM) and DαAA (200 μM) suppressed completely depolarizations induced by NMDA (10 μM). Brief HFS failed to establish LTP when the slice was perfused with a mixture of DαAA (200 μM) and DNQX (10 μM), this latter compound acting to block selectively non-NMDA receptors. Thus LTP can be established, at least partly, solely by activation of AP5-insensitive EAA receptors; raising the intriguing possibility of the existence of 2 pharmacologically distinct NMDA receptors. Supported by the Human Frontiers in Science Program.

Collingridge, G.A., Kehl, S.J. and McLennan, H., Excitatory amino acids in synaptic transmission in the Schaffer collateral-commissural pathway of the rat hippocampus., J. Physiol., 334, 33-46, 1983

407.11

DELTA OPIOID RECEPTOR BUT NOT NMDA RECEPTOR ACTIVATION IS REQUIRED TO INDUCE LTP OF SYNAPTIC TRANSMISSION IN THE LATERAL PERFORANT PATH

C.R. Bramham*[1], N.W. Milgram[2], and B. Srebro*[1]. [1]Department of Physiology, U. of Bergen, N-5009 Bergen, Norway, and [2]Life Sciences Department, U. of Toronto, Ontario M1C 1A4, Canada.

Rats were anesthetized with urethane and implanted with a stimulus electrode in either the lateral (LPP) or medial (MPP) division of the perforant path-granule cell projection, under electrophysiological control. The fascia dentata was perfused with oxygenated krebs via a push-pull cannula, while two attached electrodes recorded evoked potentials in the granule cell and molecular layers. Tetanization of the LPP or MPP elicted robust LTP in rats perfused with standard medium. In the MPP, trains given during perfusion with medium containing AP5 (50 uM), a NMDA receptor blocker, failed to produce LTP. In the LPP, however, AP5 prevented population spike potentiation without affecting the synaptic, EPSP component of LTP. Further, the opioid receptor antagonists naloxone and ICI 174,864 (delta selective; 0.1 uM), while having no effect on single-pulse transmission, blocked both EPSP and population spike LTP of the LPP. The mechanism of LTP in the LPP illustrates peptidergic control of activity-dependent synaptic plasticity in brain.

407.12

RECEPTOR MECHANISMS OF OPIOID ENHANCEMENT OF LONG-TERM POTENTIATION IN THE LATERAL PERFORANT PATH. C.W.Xie and D.V.Lewis. Pediatric Neurology, Duke Univ.Med.Center, Durham,NC27710.

Opioid effects on the induction of long-term potentiation (LTP) in the lateral perforant path (LPP) were investigated on rat hippocampal slices. High frequency stimuli (100Hz,1s) delivered to the LPP terminals in the outer molecular layer of the dentate induced LTP of the orthodromic population spike (OPS) and population EPSP slope recorded from the granule cell layer and molecular layer. Bath application of naloxone (1-10 μM), or combined application of the selective mu (β-FNA,10μM) and delta antagonist (ICI 174,864, 20μM) significantly reduced the LTP of both OPS and EPSP. β-FNA (10μM) but not ICI 174,864 (20μM) alone blocked LTP of EPSP. Either of them alone only slightly reduced LTP of OPS. The mu agonist PLO17 (0.3-1 μM) reduced the threshold and increased the amount of LTP of OPS. Its effects were completely antagonized by 1 μM naloxone and the NMDA antagonist D-APV (100μM). These findings suggest that endogenous opioids released during high frequency stimuli facilitate LTP at the LPP synapses through both mu and delta receptors. Supported by NS22170.

407.13

SACCHARIN BLOCKS THE INDUCTION OF LONG-TERM POTENTIATION (LTP) IN THE HIPPOCAMPUS. W. Morishita, S. S. Chirwa, P. May*, Z. Xie and B. R. Sastry. Neurosci. Res. Lab., Dept. of Pharmacology, The Univ. of B. C., Vancouver, Canada, V6T 1W5.

Tetanic stimulation of stratum radiatum in guinea pig hippocampal slices induces a long-term potentiation (LTP) of the population spike recorded in the CA1 region. In the present study the ability of saccharin to interfere with this induction was examined. When tested in dose ranges of 2.5 to 20 mM (applied for 5-10 min, n=6 for each dose), Na saccharin did not significantly alter the size of the population spike. When the drug (10 mM, 10 min) was applied during the tetanic stimulation (400 Hz, 0.5 s) of stratum radiatum, LTP was not observed (population spike control size: 1.11 ± 0.33 mV; 30 min after tetanus: 1.13 ± 0.47 mV, n=9). In the same slices, a subsequent tetanus given in the absence of saccharin clearly caused LTP (population spike 30 min post-tetanus: 2.84 ± 0.44 mV). The amplitude of the EPSP in Mg-free medium containing CNQX (10 μM, n=4) was not significantly altered by saccharin (10 mM). In a medium containing 2-amino-5-phosphonovalerate (25 μM, n=4), however, the EPSP was slightly decreased during saccharin application. These results indicate that the blockade of the induction of LTP by saccharin is not through an antagonism at the NMDA receptors. (Supported by grants from the Canadian MRC and Glaxo Research Labs.)

407.14

STUDIES ON ENDOGENOUS SUBSTANCES THAT INDUCE LONG-TERM POTENTIATION (LTP). Z. Xie, W. Morishita, T. Kam*, H. Maretic* and B. R. Sastry. Neurosci. Res. Lab., Dept. of Pharmacology, The Univ. of B. C., Vancouver, Canada, V6T 1W5.

Fluid samples collected during a tetanic stimulation of the rabbit neocortical surface, cause LTP in guinea pig hippocampal slices. In the present study, these samples were separated into various fractions according to molecular weights. Applications of 2 ml of <3, 3-10 or >50 kD, but not of 10-25 or 25-50 kD, fractions on guinea pig hippocampal slices induced LTP (stratum radiatum stimulation-induced population spike in the CA1 area recorded 60 min after drug application as a % of control: <3 kD: 127.75 ± 10.65, n=10; 3-10 kD: 136.875 ± 9.11, n=8; 10-25 kD: 93.13 ± 20.45, n=8; 25-50 kD: 101.38 ± 9.99, n=8; >50 kD: 155.25 ± 10.25, n=8). The EPSP was potentiated without a significant change in the membrane potential and input resistance (n=6). 2-Amino-5-phosphonovalerate (25 μM) did not block the induction of LTP by these substances (n=6). Gel-electrophoresis (1 & 2 D) of the samples revealed the presence of peptides. Samples collected from rabbits pretreated with MK-801 (0.5 mg/kg ip) did not induce LTP (n=6). Tetanic stimulation in hippocampal slices, however, induced LTP in the presence of samples from rabbits pretreated with MK-801 (n=6). These results suggest that the release, but not the LTP-inducing effect, of the endogenous substances involves NMDA receptor activation. (Supported by grants from the Canadian MRC and Glaxo Res. Labs.)

407.15

SYNAPTIC PLASTICITY IN THE AVIAN HIPPOCAMPUS. A.Wieraszko, G.F. Ball, Department of Biology and Department of Psychology, Boston College, Chestnut Hill, MA 02167.

Long-term potentiation (LTP) is a stimulation-evoked, persistent increase in synaptic efficiency. LTP, which has been considered to be a neuronal mechanism underlying memory is most pronounced in the hippocampus. Although there has been recently a great deal of interest in the hippocampus and memory in birds, LTP has not yet been described in the avian hippocampus. We present here studies on synaptic plasticity and LTP in avian hippocampus. Extracellular recordings from hippocampal slices of female song sparrows (Melospiza Melodia) were taken using standard techniques. The evoked responses were much smaller and less stable as compared to those found in the mammalian hippocampus. These responses were calcium-dependent proving their synaptic origin. Frequency potentiation could not be demonstrated. Paired-pulse facilitation showed inhibition of the second response at latencies below 20 ms and facilitation at 30 ms. Train stimulation with 20 Hz evoked a stable increase in the size of the evoked response lasting approximately two hours. This increase was classified by us as LTP. These data indicate that the avian hippocampus possesses several properties typical for the mammalian hippocampus including a most intriguing one-LTP.

Supported by NSF grant BNS 8604955 and NIH grant NS 27866-01.

NEURONAL DEATH: LESION STUDIES

408.1

MORPHOMETRIC ANALYSIS OF THE CEREBELLAR NUCLEI IN LURCHER MUTANT MICE. J. A. Heckroth, Terre Haute Center for Medical Education, Indiana University, Terre Haute, IN 47809.

Mutant gene expression in lurcher mutant mice causes the postnatal cell death of the entire population of cerebellar Purkinje cells. The cerebellar nuclei, while reduced in size, are reported to contain a normal number of neurons (Caddy and Biscoe (1979), Phil Trans Roy Soc Lond B, 287:167). The volume of the cerebellar nuclei in normal and lurcher mice has been estimated utilizing planimetric measurements of evenly spaced coronal sections. In both normal and lurcher mice the interposed nucleus comprises about 50% of the total nuclear volume, with the fastigial and dentate nuclei representing about 25% each. The lurcher fastigial and interposed nuclei are each reduced in volume by about 65%, while the dentate nucleus is reduced by only 53%. The volume fraction (V_V) of the nuclei occupied by neuronal somata and by neuropil has been estimated by the stereological point counting method. Neuronal somata occupy about 10% of the volume of the normal interposed and fastigial nuclei, and about 20% of the homologous subdivisions in lurcher. The volume fractions, combined with the total volume estimates, suggest that in the lurcher interposed and fastigial nuclei the total volume of neuronal somata is reduced by about 25% from normal, while the volume of neuropil is reduced by about 75% from normal. The relatively large reduction in the volume of neuropil may be attributed to the absence of Purkinje cell axons and terminals, and a reduction in the dendritic arbors of nuclear neurons. The neuronal volume reduction is apparently the result of smaller neuronal somata in lurcher. Further stereological measurements to determine the individual contributions of axonal, terminal, and dendritic losses to the total reduction of the lurcher cerebellar nuclear neuropil are underway.

408.2

IMMUNOLOGICAL ACTIVATION OF GLIAL CELLS IN EARLY POSTNATAL RATS AFTER SCIATIC NERVE CRUSH. W.J. STREIT and T. MORIOKA. Depts. of Neuroscience and Neurological Surgery, University of Florida, Gainesville, FL 32610.

We have examined the glial reaction accompanying motor neuron death following sciatic nerve crush in the newborn rat using a panel of monoclonal antibodies directed against brain macrophage antigen (ED2) and various immunomolecules, i.e. Ia antigen (OX-6), complement receptor (OX-42), CD4 antigen (W3/25), and leucocyte common antigen (OX-1). These antigens were found to be mostly absent from the normal neonatal rat CNS, but their rapid appearance, except for ED2, was stimulated after sciatic nerve crush in the neuropil around spinal motor neuron degeneration. Cell types expressing immunomolecules within days after axotomy were perivascular and microglial cells, and thus like in the adult. However, while in the adult the expression of immunomolecules on these cell types takes a gradual and prolonged time course lasting several months, in the neonate enhanced immunoreactivity at the lesion site could no longer be detected 2 weeks after nerve section. Our results therefore show that immunocompetent cells are present in the immature rat CNS, but their activation is of more rapid onset and briefer duration than in the adult. (Supported by NIH/NINCDS PO1NS27511).

408.3

ONSET OF REACTIVE ASTROGLIOSIS IN THE LATERAL GENICULATE NUCLEUS (LGN) OF INFANT AND ADULT CATS. R.E. Kalil. Center for Neuroscience, Univ. of WI, Madison, WI 53706

Following a lesion of visual cortex, LGN neurons undergo retrograde degeneration, which is accompanied by a series of responses in local astrocytes, known as reactive astrogliosis. One outcome of this reaction is that astrocytes become phagocytic and remove neuronal debris. Previously, we (Kalil et. al.,'89) compared the time course of reactive gliosis at birth or in adulthood by using a monoclonal antibody against glial fibrillary acidic protein (GFAP) to monitor the intensity of the gliotic reaction at survival times that ranged from 1 day to 26 wks.

This work now has been extended by combining GFAP immunocytochemistry with electron microscopy to focus on the earliest reactions of LGN neurons and astrocytes to a unilateral lesion of visual cortex made in newborns or adults. In neonates, GFAP+ astrocytes are evident in the LGN at 1 day postlesion; neurons in advanced degeneration are seen at 3 days. However, this sequence possibly is confounded by the fact that LGN astrocytes on the unoperated side of the brain also are GFAP+ in neonates. By contrast, normal LGN astrocytes in adult cats rarely are positive for GFAP, but display strong immunoreactivity at 1 day postlesion, before degenerating neurons are observed. These results suggest, therefore, that reactive astrogliosis in the LGN may be triggered by a signal that precedes the onset of overt neuronal degeneration.

408.4

RESPONSES OF BRAIN MACROPHAGES TO INDUCED CELL DEATH IN THE VISUAL SYSTEM OF NEONATE AND ADULT RATS. C.E.Milligan, P.Levitt and T.J.Cunningham. Dept. of Anatomy. Medical College of PA, Philadelphia, PA 19129.

The response of brain macrophages (BMOs) to induced cell death was examined in neonate and adult Long Evans rats. Lesions to the visual cortex of infant and adult rats induce cell death in the ipsilateral dorsal lateral geniculate nucleus (dLGN). BMOs are localized using the ED-1 monoclonal antibody antigen, which recognizes all cells of the rat monocyte/macrophage lineage, but not microglia. At no time throughout normal postnatal development or in the normal adult are BMOs localized in the dLGN. After infant lesions, the entire nucleus degenerates completely by four days. BMOs are first observed among numerous pyknotic cells 48 hours following the lesion. They are large round cells and reach maximum levels four days after the lesion, completely occupying the nucleus. In adults, similar lesions to the visual cortex result in a much more protracted period of degeneration. Furthermore, subpopulations of cells located in different regions of the nucleus degenerate at different rates. The timing of appearance and location of BMOs in the dLGN correspond to the spatiotemporal pattern of degeneration in the nucleus. However, the cells are fusiform shaped and highly branched, unlike those found after infant lesions. These results suggests that the timing of BMO invasion is strictly controlled by degenerating elements. The acquisition of distinct morphologies may reflect the constraints imposed by the brain parenchyma on their invasion at different ages. (Supported by NS16487 from NINCDS.)

408.5

STUDIES RELATED TO THE USE OF COLCHICINE AS A NEUROTOXIN IN THE SEPTOHIPPOCAMPAL CHOLINERGIC SYSTEM: I. SPECIFICITY OF ACTION. S. R. Ginn and G. M. Peterson. Dept. of Anatomy and Cell Biology, East Carolina University School of Medicine, Greenville, NC 27858.

Previous research has indicated that, in the medial septum, colchicine is toxic to neurons immunoreactive for choline acetyltransferase (ChAT), but not glutamic acid decarboxylase (GAD). In addition, a profound deficit in acetylcholinesterase- (AChE) positive fibers is observed in the hippocampal formation, especially in the dentate gyrus and the temporal pole of the hippocampus. Here we report the results of several studies designed to more fully elucidate this colchicine-induced toxicity in the septohippocampal cholinergic system. Except as indicated below, female Sprague-Dawley rats (200-250 g) received intracerebroventricular (ICV) injections of 2.5 μl 0.4% colchicine in saline unilaterally into the right lateral ventricle, adjacent to the body of the fornix. Following a survival period of 1, 2 or 3 weeks the rats were perfused through the ascending aorta with saline followed by ice-cold 4% paraformaldehyde in phosphate buffer (pH 7.4). Brain tissue was cut on a sliding microtome in a 1-in-5 series throughout the septum and hippocampus. Colchicine significantly reduced the numbers of choline acetyltransferase- (ChAT) immunoreactive neurons in the medial septum (MS) at all survival times, and the effect appeared to be progressive (i.e., more cell loss occurred at longer survival times). In addition, the pattern of AChE fiber staining in the hippocampus was disrupted in a time-dependent manner. However, even at the longest survival period, GAD-immunoreactive neurons in the medial septum were unaffected by ICV colchicine injections. Injections of colchicine (0.4% in 1 μl saline) confined to the body of the fornix produced effects similar to ICV colchicine injections: i.e., a reduction in the number of ChAT-immunoreactive neurons in the medial septum and AChE-positive fibers in the dentate gyrus and hippocampus. The results of these experiments indicate that colchicine is an effective and specific neurotoxin for septohippocampal cholinergic neurons when injected into either the lateral ventricles or the fimbria-fornix.

408.6

STUDIES RELATED TO THE USE OF COLCHICINE AS A NEUROTOXIN IN THE SEPTOHIPPOCAMPAL CHOLINERGIC SYSTEM. II. MECHANISM OF ACTION. G. M. Peterson and S. R. Ginn. Dept. of Anatomy and Cell Biology, East Carolina University School of Medicine, Greenville, NC 27858.

Colchicine disrupts microtubules by binding free tubulin, resulting in the blockade of axoplasmic transport. To determine if this mechanism of action mediates colchicine-induced toxicity of septohippocampal cholinergic neurons, the fluorescent dye Fluoro-Gold (50 nl 2% FG) was injected into 2 sites within the ventral hippocampus. Thirty minutes later 2.5 μl of 0.4% colchicine or its inactive isomer, lumicolchicine, was injected into the right lateral ventricle. One week later brains were fixed by perfusion with paraformaldehyde and cut on a sliding microtome throughout the medial septum and hippocampus. Both FG- and ChAT-labeled cells were significantly reduced in the medial septum following colchicine injections, but were not affected by lumicolchicine, indicating that colchicine does disrupt axoplasmic transport mechanisms in septohippocampal neurons, and that this loss of transport is related to the toxic effect. In a related study the effect of colchicine on the transport of nerve growth factor (NGF) was examined. It is thought that the loss of cholinergic neurons in the medial septum results from the colchicine-induced blockade of the axoplasmic transport of NGF from the hippocampus. In support of this hypothesis we have found that hippocampal levels of NGF increase 200% over controls 2 weeks following colchicine injections (ELISA of NGF conducted by Dr. K. A. Crutcher at the Univ Cincinnati Med Ctr). As an additional test of this hypothesis, ^{125}I-NGF was injected bilaterally into 2 sites in each hippocampus and 2.5 μl 0.4% colchicine was injected into the right lateral ventricle. Twenty-four hours later paraformaldehyde-fixed brains were embedded in parafin, cut on a rotary microtome and processed for autoradiography. The number of radiolabeled neurons was dramatically reduced in the medial septum ipsilateral to the injection indicating that colchicine blocked the transport of ^{125}I-NGF. Thus, these studies suggest that colchicine is toxic to septohippocampal cholinergic neurons because it blocks the axoplasmic transport of nerve growth factor.

408.7

PROTEOLYSIS OF BRAIN SPECTRIN PRECEDES THE DEGENERATION OF SEPTAL NEURONS FOLLOWING FIMBRIA-FORNIX TRANSECTION. J.M. Roberts-Lewis, C. DiCocco*, S.L. Meyer, M.E. Lewis and R. Siman. Cephalon, Inc., West Chester, PA 19380.

Acute injuries or degenerative diseases of the CNS result in the structural degradation of damaged neurons, and synaptic reorganization of surviving neurons. These morphological changes have been attributed to the activity of proteases in the CNS. For example, the calcium-dependent proteolysis of brain spectrin (fodrin) by calpain I has been proposed to constitute a requisite step in the intracellular cascade by which excitatory amino acids induce neuronal death. In order to evaluate the possible role of spectrin proteolysis in mediating the death of septal neurons after transection of the fimbria-fornix, we have used immunoblot analysis to measure spectrin breakdown in homogenates from septum and hippocampus at 1, 7 and 14 days after unilateral transection of the fimbria-fornix. Spectrin breakdown products were detected in the septum, but not hippocampus, at 7 and 14 days after transection of the fimbria-fornix. There was no evidence of spectrin breakdown in either septum or hippocampus 24 hours following fimbria-fornix transection. The appearance of spectrin breakdown in the septum occurs before any apparent structural degeneration of septal perikarya. These findings suggest that proteolysis of this cytoskeletal protein may be an early event in the cascade that ultimately leads to the destruction of these neurons in the absence of NGF. We are currently evaluating the effects of NGF treatments on the appearance of spectrin breakdown in the septum after fimbria-fornix transection.

408.8

CORTICAL CYTOLOGY IMPROVES QUANTITATIVELY IN INFANTILE HYDROCEPHALIC ANIMALS AFTER SURGICAL DECOMPRESSION. J.P. McAllister, L.M. Sharpe* and P.M. Hale.* Depts. Anatomy & Neurosurgery, Temple U.Sch. Med., Philadelphia, PA 19140.

As a sequel to our previous descriptive analyses, the present study has quantified the effects of ventriculomegaly on the immature cerebral cortex. Hydrocephalus was induced in 4-11 day old kittens by intracisternal injection of kaolin; some hydrocephalic animals received ventriculoperitoneal shunts 10-14 days post-kaolin. Hydrocephalic animals (n=4) were sacrificed at 16-44 days post-kaolin and shunted animals (n=3) at 11-30 days post-shunt. Nissl-stained sections from cortical areas 17 and 22 were analyzed light microscopically with Bioquant software. Compared to saline-injected controls (n=5), cortical thickness in hydrocephalics decreased 80% and 70% in areas 17 and 22, respectively. The number of neurons per 10 sq.μm increased 27-33% in laminae V-VI and 12-55% in layers II-IV. A consistent 35-48% decrease in soma size was most pronounced in layers V-VI. After shunting cortical thickness and soma size returned to near or above control levels; neuronal density returned either to control levels or remained about 14% low. More improvement was found in area 17 than 22. These results indicate that hydrocephalus causes severe alterations in cortical cytology but that shunting can promote neuronal recovery. Supported by HD21527 to JPM.

408.9

MORPHOLOGY OF CAUDATE NUCLEUS CHANGES AFTER PRENATAL UNILATERAL NEOCORTICAL LESION.
L.D. Loopuijt*, J.R. Villablanca and D.A. Hovda, Mental Retardation Research Center, UCLA, Los Angeles, CA 90024-1759.

4 Fetal cats (E43-48) received a small lesion in frontal (3x) or parietal (1x) cortex and were sacrificed as young adults. Coronal, thionin stained sections of these brains were used for calculation of caudate volume, determination of neuron and glia number per unit volume and measurement of surface areas of neuron somas by means of computerized morphometrics. Compared to weight matched controls (N=2), the volume of the ipsilateral caudate (being 8-13% larger than the contralateral) was larger by 36-42%, whereas the contralateral caudate was 20-30% larger. The cell packing density was similar for both sides, except for 1 cat, where the density was higher ipsilateral (P 0.05, sign test). In 2 cats the average neuronal soma area was 15-30% smaller (P 0.05, t-test) in the ipsilateral, compared to the contralateral caudate. The increase in caudate volume with preservation of cell packing density means that the caudate contains a larger amount of cells. It contrasts with the shrinkage in volume of thalamus and cortex. (Soc Neurosci Abstr 13(87)1116). Grants HD-05958, HD-04612.

408.10

ANTEROGRADE TRANSPORT IN AXOTOMIZED CORTICOSPINAL NEURONS.
M.K. Garver, R.L. McBride and E.R. Feringa. Dept. of Neurology, Medical College of Georgia and Dept. of Veterans Affairs Med. Ctr., Augusta, GA 30910.

We measured slow and fast anterograde transport in proximal corticospinal axons 5, 10, and 20 weeks after complete T-9 spinal cord transection. We studied slow transport in three groups of 10 transected and 10 control rats each. 100 μCi of ^{3}H proline were injected into motor cortex 3, 8 or 18 weeks following cord transection; 14 days later the rats were perfused and a 5 mm segment of spinal cord (15 mm from the obex) was removed, solubilized, and scintillation counted. For fast transport, proline was injected 5 or 10 weeks after transection followed by perfusion 24 hours later.

Slow transport in transected rats vs controls was increased at 5 weeks (5990±757 DPM [mean±SEM] vs 3576±363, $p<0.01$), but was normal at 10 weeks (5136±933 vs 6452±1385) and at 20 weeks (2312±243 vs 2430±621). Fast transport in transected rats was not significantly different from controls at either 5 weeks (3087±402 vs 2677±353) or 10 weeks (2681±343 vs 2843±146). We conclude that slow transport is increased at 5 weeks, and is normal at 10 and 20 weeks. Fast transport is not changed 5 or 10 weeks after cord transection.

Supported by the Dept. of Veterans Affairs and the Medical College of Georgia.

408.11

AXOTOMIZED CORTICOSPINAL NEURONS CANNOT BE RETROGRADELY LABELED WITH FLUORO-GOLD ONE YEAR AFTER SPINAL CORD TRANSECTION. R.L. McBride, W.B. Harper* and E.R. Feringa. Dept. of Veterans Affairs Med. Ctr. and Dept. of Neurology, Medical College of Georgia, Augusta, GA 30910.

Corticospinal tract neurons axotomized by T-9 spinal cord transection lose their ability to be retrogradely labeled with horseradish peroxidase; at 10 weeks after transection, 25% can be labeled, and by one year, only 7%. In contrast, at 20 weeks after transection, retrograde labeling of these neurons with Fluoro-Gold is unimpaired. To determine the long term effects of axotomy on retrograde labeling with Fluoro-Gold, we transected the spinal cord of 10 7-week-old female rats at T-9. One year later, a cotton pellet soaked in Fluoro-Gold was placed into a new transection site at T-1; matched controls were treated similarly. Four days later, the rats were perfused. The mean number of labeled corticospinal neuronal somata was 1919±93 (mean ± SEM) in controls and 1337±137 in transected rats, a decrease of 30% ($p<0.01$). The loss of labeling was confined to the cortical area representing the distribution of hindlimb upper motoneurons. This study provides further evidence for long-term, gradual deterioration of axotomized corticospinal neurons. Supported by the Dept. of Veterans Affairs and Medical College of Georgia.

408.12

ONE YEAR AFTER SPINAL CORD TRANSECTION, RETROGRADE LABELING OF RUBROSPINAL NEURONS WITH FLUORO-GOLD IS DECREASED. W.B. Harper*, R.L. McBride and E.R. Feringa. Dept. of Veterans Affairs Med. Ctr. and Dept. of Neurology, Medical College of Georgia, Augusta, GA 30910.

One year after a T-9 spinal cord transection, fewer corticospinal neurons can be retrogradely labeled with Fluoro-Gold from the T-1 level than in control rats. To determine if a similar decrease occurs in axotomized rubrospinal neurons, we assessed the number and size of red nucleus neurons which could be labeled with Fluoro-Gold. We transected the spinal cord at T-9 in ten seven-week-old female rats. After one year, we placed a cotton pellet soaked with Fluoro-Gold into a new transection site at T-1. Four days later, the rats were perfused. Non-transected matched controls were treated similarly. The red nucleus is topographically organized, with the caudal portion projecting primarily to the lumbar spinal cord. We found a significant decrease (29%, $p<0.05$) in the number of Fluoro-Gold-labeled neurons in the caudal part of the nucleus in transected rats compared to controls, but no significant change in the rostral part. Labeled neurons rostrally were 19% smaller in transected rats; caudally, neurons were 40% smaller. The decrease in number of rubrospinal neurons labeled with Fluoro-Gold is comparable in magnitude to the decrease in labeled corticospinal neurons. Supported by DVA and MCG.

408.13

DEATH BY DEAFFERENTATION: THE ROLE OF MITOCHONDRIA IN AUDITORY NEURON SURVIVAL FOLLOWING COCHLEA REMOVAL. G. E. Hyde and D. Durham. Departments of Otolaryngology and Biological Structure, RL-30, University of Washington, Seattle, WA 98195.

Removal of excitatory input to second-order auditory neurons (n. magnocellularis, NM) in the chick brain stem results in the death of ~20% of the neurons at 5 days survival. In a study of ultrastructural changes in NM neurons following cochlea removal, we found a dramatic proliferation of mitochondria in the majority of ipsilateral NM neurons (Hyde & Durham, Soc. Neurosci. Abstr. *15*:290, 1989). However, we found a subpopulation of NM neurons with early degenerative changes which did not show mitochondrial proliferation. To test the hypothesis that mitochondrial proliferation is necessary for neuronal survival following cochlea removal, we used chloramphenicol, a mitochondrial protein synthesis inhibitor, to block mitochondrial proliferation after cochlea removal in 2-week-old chicks.

We administered chloramphenicol at the rate of 1000 mg/kg/day for either 6, 12, or 24 hours following cochlea removal. After 5 days survival, animals were deeply anesthetized and sacrificed. NM neuron counts were made in 10μm Nissl-stained paraffin sections; the NM neurons contralateral to cochlea removal serving as a within-animal control. In animals treated 6 hours with chloramphenicol, we found no enhanced neuronal death ipsilateral to cochlea removal compared to vehicle-treated animals ($p>.60$). However, in animals treated for either 12 or 24 hours with chloramphenicol, we found a significant increase in ipsilateral neuronal death (65% loss, $p<.05$). Thus, we conclude that mitochondrial proliferation becomes critical to auditory neuron survival between 6 and 12 hours after the removal of excitatory input. (Supported by PHS grants DC00018 and DC00520 and the Deafness Research Foundation)

408.14

PREDICTING INDIVIDUAL VARIATIONS IN DEGENERATION OF HIPPOCAMPAL DENTATE NEURONS FOLLOWING ADRENALECTOMY

Edward J. Roy, Diane M. Lynn,* and Charles W. Bemm*
Psychology Dept., University of Illinois, Champaign, IL 61820

Corticosterone appears to have two markedly different effects on cells of the hippocampus in rats. On one hand, elevated levels of corticosterone contribute to the degeneration of pyramidal cells. On the other hand, elimination of corticosterone by adrenalectomy may cause degeneration of dentate granule cells (Sloviter et al., 1989). However, the latter response is highly variable. Low levels of corticoids from accessory adrenal tissue not detectable by radioimmunoassay may provide sufficient hormone to maintain granule cell viability. We describe simple measures that predict which individual adrenalectomized rats have degeneration of the granule cell layer. Body weight gain after adrenalectomy is correlated with granule cell layer area at sacrifice three months after surgery ($r = 0.747$, $n = 15$, $p < 0.001$). Also, short term loss of body weight when saline drinking water is replaced with tap water predicts the degree of degeneration of the granule cell layer ($r = -0.642$, $n = 15$, $p < 0.001$). The maximal effect we observed was a 64% reduction in the area of the granule cell layer. These observations may aid further study of this striking effect of adrenal hormones on brain anatomy.

DEVELOPMENT AND PLASTICITY—VISUAL SYSTEM: MOLECULAR AND CELLULAR MECHANISMS II

409.1

VISUALLY INNERVATED AUDITORY CORTEX IN FERRETS GENERATES VISUAL RESPONSE PROPERTIES SIMILAR TO THOSE IN NORMAL VISUAL CORTEX. A. W. Roe, S. L. Pallas, Y. H. Kwon, and M. Sur. Dept. of Brain & Cognitive Sci., M.I.T., Cambridge, MA 02139.

We are examining how a cortical area of one sensory modality might process sensory input of another modality, by routing visual inputs to primary auditory cortex (A1) in ferrets. In neonatal ferret pups, normal retinal targets (LGN and SC) are removed and the auditory thalamus deafferented. Retinal fibers consequently innervate the auditory thalamus and thereby provide visual input to A1.

We have recorded electrophysiologically the visual response properties of single cells in A1 of lesioned animals reared to adulthood, and compared in detail the responses with those in primary visual cortex (V1) of normal animals. Visual units in A1 have longer latencies to optic chiasm stimulation (n=390, $\bar{x}$=7.0 msec) compared to cells in V1 (n=93, $\bar{x}$=4.9), a finding consistent with our previous observation that retinal input to the auditory pathway arises primarily from retinal W cells (Sur et al., Science 242:1437, 1988). Visual units have larger receptive fields in A1 ($\bar{x}$=11.6°) than in V1 ($\bar{x}$=4.4°) and generally poorer responsiveness. Ocular dominance in both V1 (n=57) and A1 (n=88) is highly skewed towards the contralateral eye. The proportions in A1 of simple (32%), complex (28%), and non-oriented (40%) cells are similar in general to those in V1. Of a smaller population of cells (n=17 in A1, 15 in V1) examined quantitatively, the degree of orientation tuning and orientation tuning widths are very similar, as is the degree of direction tuning.

These results demonstrate that following specific neonatal lesions, auditory cortex can produce visual cortical receptive field properties similar to those found in normal visual cortex. Our data are consistent with the interpretation that sensory cortices normally employ stereotypical circuits to perform equivalent transformations on the input they receive, or perhaps that modality-specific circuits can be induced in cortex by afferents during development.

Supported by EY07719, the McKnight Foundation, and a Whitaker Fellowship.

409.2

CORTICAL BINOCULAR INTERACTIONS IN NORMAL AND STRABISMIC MONKEYS. J. Ni*, E. L. Smith III, Y. M. Chino, K. Kitagawa*, M. L. J. Crawford. College of Optometry, University of Houston, TX 77204-6052.

Previous studies have demonstrated that experimentally induced strabismus causes dramatic alterations in cortical ocular dominance in young monkeys. When monocular stimulation techniques are employed, the majority of cortical neurons respond exclusively to one eye. However, strabismic humans exhibit robust inhibitory binocular interactions. The purpose of this study was to determine if any binocular interactions could be revealed in the striate cortices of strabismic monkeys when stimuli are presented to both eyes simultaneously.

Extracellular, microelectrode recording techniques were used to investigate the sensitivity of cortical neurons to the relative binocular spatial phase of dichoptic grating stimuli (Ohzawa and Freeman, 1986). In normal monkeys (n=5), the responses of simple cells varied systematically with relative spatial phase; the disparity tuning functions typically included both facilitatory and inhibitory responses. Some complex cells also exhibited phase specific responses. However, in most cases complex cells were not selective for relative binocular phase, but their binocular responses were larger than either monocular response. Our studies of monkeys reared with an optically induced strabismus (n=3), revealed two major differences. In addition to an increase in the number of neurons that demonstrated no binocular interactions, there was a substantial increase in the proportion of neurons that demonstrated inhibitory responses under binocular conditions at all spatial phases.

409.3

DEVELOPMENT OF NEURAL CONNECTIONS BETWEEN TRANSPLANTED LATERAL GENICULATE NUCLEUS AND HOST VISUAL CORTEX IN THE RAT. T. Kurotani*, N. Yamamoto* and K. Toyama. Department of Physiology, Kyoto Prefectural University of Medicine, Kawaramachi-Hirokoji, Kamigyo-ku, Kyoto 602, Japan.

Development of neural connections between the transplanted fetal lateral geniculate nucleus (LGN) and the neonatal host visual cortex (VC) was studied electrophysiologically in VC slices including transplanted LGN. Current source density (CSD) analysis of the field potentials and study of intracellular responses in VC under LGN stimulation indicated that monosynaptic connections from LGN to VC cells were established broadly in layers II-VI, while polysynaptic connections remained poorly developed in preparations of 1 week after transplantation. LGN stimulation also produced antidromic responses in a large fraction (57%) of VC cells in layers II-VI. In preparations of more than 3 weeks after transplantation, monosynaptic connections were restricted to layers IV and VI, while polysynaptic connections appeared in layers II-III and V. Efferent cells projecting to LGN became less numerous (18%) and restricted to layers V and VI. Corresponding results were obtained by morphological study using antero- and retrograde labelling with a fluorescent dye (DiI, Molecular probe). In summary, development of afferent and efferent connectivities in transplant preparations was characterized by formation of transient excessive projections and succeeding selection of the appropriate connections.

409.4

Long-term potentiation and depression in kitten visual cortex studied *in vivo*. H. Tamura*, Y. Hata and T. Tsumoto. Dept. Neurophysiol., Biomed. Res. Ctr., Osaka Univ. Med. Sch., Kitaku, Osaka, 530 Japan.

Long-term potentiation (LTP) or depression (LTD) of visual cortical responses to stimulation of the optic nerve (ON) could be induced by tetanic stimulation of ON of the same or the other side, respectively, in kittens during "critical period" of postnatal development (Tsumoto and Suda, Brain Res., 168, 190, 1979). In the present study, we addressed a question of what conditions are optimal to induce such homosynaptic LTP and heterosynaptic LTD, in 4-8 week-old kittens anesthetized with N_2O and Halothane. Extracellular field potentials to test shocks applied alternatively to each optic nerve at 0.1 Hz were recorded simultaneously from the primary visual cortex of both hemispheres. Tetanic stimulation applied to ON at 5 Hz for 1 or 5 min failed to induce LTP but longer tetanus (15-60 min) could induce LTP and LTD. Since afferent spontaneous activity was suggested to play a role in visual cortical plasticity (Stryker and Harris, J. Neurosci., 6, 2117, 1986), retinal neuronal activities were blocked by an intraocular injection of tetrodotoxin (TTX). After the TTX injection, homosynaptic LTP could more easily be induced but heterosynaptic LTD could not be induced at all. These results suggest that afferent spontaneous activity may play a role in induction of LTP and LTD in kitten visual cortex.

409.5

NON-HEBBIAN SYNAPSES IN RAT VISUAL CORTEX. A.Kossel (1), T.Bonhoeffer (2) and J.Bolz (1). (1) Friedrich-Miescher Labor der Max-Planck Gesellschaft, and (2) Max-Planck Institut für biologische Kybernetik, 7400 Tübingen, FRG.

It has been demonstrated experimentally that synaptic connections in the visual cortex can be modified by correlated activity of pre- and postsynaptic cells, as predicted by a classical model proposed by Hebb. These studies also found that the synaptic enhancement was restricted to only those inputs which were active simultaneously with the postsynaptic cell. We investigated whether the enhancement remains also spatially confined to only those postsynaptic neurons which had been coactivated with the presynaptic fibers.

Double intracellular recordings were performed in slices from the visual cortex prepared from 2-4 week old rats. The two cells were lying close to each other (distance<200 μm), but they were not synaptically connected. Stimulating electrodes were placed in the white matter and short current pulses (ISI = 4sec) were applied to yield subthreshold EPSPs in both cells. Synaptic enhancement was induced by pairing stimulation of the input fibers with intracellular depolarization of one of the two recorded cells. The other cell was not depolarized. In all 4 out of 15 experiments, in which we could potentiate the synaptic responses of the paired cell, the responses of the second cell were also reinforced. Control experiments with a third extracellular recording electrode showed that the strengthening does not extend throughout the whole slice. Our results indicate that in contrast to the Hebbian rule, synaptic enhancement is not restricted to the paired cell, but spreads to neighboring neurons. This effect could be relevant during the development of the cortex: adjacent cells will tend to assimilate their functional properties. This in turn might lead to clustering of cells with similiar response properties, a common organizing principle of the cortex.

409.6

SYNAPTOGENESIS COMPLEMENTS ASSOCIATIVE SYNAPTIC MODIFICATION IN A MODEL OF THE DEVELOPMENT OF OCULAR DOMINANCE. C.M. Colbert, S.M. Friedman*, and W.B Levy Dept. of Neurosurgery, Box 420 Med. Ctr., Univ. of Virginia, Charlottesville, VA 22908.

Experience plays a central role in establishing neuronal properties and connectivity in the developing nervous system. In most activity-based neural network models, activity shapes the network through associative modification of existing synapses. Here we report on a model in which activity governs the formation of synapses as well as modification of existing synapses. The rules governing synaptogenesis are different than the associative modification rules. The synaptogenesis rules are based on both physiological and anatomical investigations in a number of systems, including the neuromuscular junction, the superior cervical ganglion, the lateral geniculate nucleus, the superior colliculus, and cerebral cortex. These studies imply that distinct presynaptic and postsynaptic mechanisms, in tandem, govern synaptogenesis. Once a synapse is formed, its efficacy is governed by a form of associative synaptic modification that has been observed in the hippocampus and in visual cortex.

To verify the validity of the model, we simulated the development of ocular dominance in the kitten under normal and experimental conditions including 1) strabismus, 2) monocular visual deprivation, 3) monocular visual deprivation with muscimol infused into visual cortex, and 4) monocular visual deprivation with APV infused into visual cortex. The simulations produced histograms of ocular dominance consistent with experimentally observed distributions.

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409.7

EFFECTS OF APV AND NMDA ON TECTAL CELL ACTIVITY IN XENOPUS. W.J. Scherer* & S.B. Udin. Dept. of Physiol., SUNY, Buffalo, NY 14214.

Chronic blockade of NMDA receptors with APV prevents matching of binocular tectal maps in eye-rotated Xenopus during the critical period, and chronic infusion of NMDA restores this plasticity in post-critical period Xenopus. In order to assess whether APV or NMDA produce these effects by altering tectal cell firing activity, we have tested the output of a subset of tectal axons which project to the nucleus isthmi. We treated one tectal lobe with a drug and recorded responses to light flashes from single, crossed isthmo-tectal units in the opposite tectal lobe. Chronic treatment with the NMDA antagonist APV did not alter tectal cell activity in critical period Xenopus when compared to age-matched controls. Chronic NMDA treatment in post-critical period animals was found to increase tectal cell firing frequency by an average of 33% when compared to age-matched controls. No difference in tectal cell firing frequency was observed between untreated critical period and post-critical period animals. These data indicate that the effects of APV on blocking plasticity are not due to a nonspecific decrease in visually evoked tectal activity. They also indicate that the restorative effects of NMDA on tectal plasticity may be related to an increase in tectal sensitivity, but do not support the hypothesis that normal loss of plasticity is correlated with diminished rates of tectal cell firing.

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409.8

PHYSIOLOGY AND MORPHOLOGY OF NEURONS IN *RANA PIPIENS* TECTAL SLICES. P. W. Hickmott and M. Constantine-Paton. Dept. of Biology, Yale Univ., New Haven, CT 06511

Work in our lab has implicated the N-methyl-D-aspartate (NMDA) receptor in the activity-dependent refinement of the retinotectal map in *Rana pipiens*. Thus, we have examined the physiological effects of various drugs, particularly those that interact with the NMDA receptor, on the evoked potential in *R. pipiens* optic tectum (Soc. Neurosci. Abstr., 15:979, 1989). We have now begun to examine the responses of single tectal neurons in a tectal slice preparation.

We have successfully recorded from tectal neurons with resting potentials ranging from -20 to -70 mV, and input impedance in the 200-400 MΩ range. In response to depolarizing voltage steps, we observe large transient inward currents, followed by sustained outward currents, which correspond to action potentials. By stimulating in the optic tract we can record large, long-lasting currents that appear to be EPSCs generated by the opening of a cation channel with a reversal potential of around 0 mV; at negative holding potentials we frequently observe large, rapid inward currents reflecting action potential firing in the cell. In some cells, instead of this EPSC, we have observed a second type of PSC with a reversal potential of about -45 mV. In some cells, we also find frequent, small, spontaneous currents that reverse at the same level as the PSC.

We have also directly demonstrated the presence of NMDA receptors on some cells; bath-applied APV, a specific NMDA receptor antagonist, reversibly blocks a slow component of the EPSC. Furthermore, low concentrations of NMDA completely block the entire PSC and appear to decrease the cell's impedance.

Finally, we have begun to examine the morphology of cells using intracellular filling with biocytin. During the introduction of biocytin cells have normal resting potentials, input impedance, and can support action potentials. All cells labelled so far have been visualized with FITC-conjugated avidin and lie in layers 4 and 6; they appear to be small pyriform cells with fine processes that can extend for many microns in the plane of section. Supported by NIH EY 06039.

409.9

POSTNATAL DEVELOPMENT OF Ca++/CALMODULIN DEPENDENT KINASE II IN KITTEN VISUAL CORTEX.

W. G. Jia, C. Beaulieu, Y.L. Liu, M. Kennedy* and M. Cynader. Department of Ophthalmology, UBC, Vancouver, B.C., Canada. *Div. Biology 216-76, CALTECH, Pasadena, CA.

A monoclonal antibody against Calcium/calmodulin dependent protein kinase II (CAM II) was utilized to localize the kinase in developing kitten visual cortex. CAM II was first expressed in deeper cortical layers (V and VI) at postnatal day 0-4 and gradually appeared in all the other layers within the first two weeks. Expression of the kinase was lost in cells of layer V and upper layer VI at about 30-40 days of age and by postnatal day 90, CAM II immunoreactivity was concentrated in layers II, IV and deeper layer VI. This laminar pattern remained constant into adulthood. EM studies showed that the kinase was found in both pre- and post-synaptic locations, particularly on synaptic vesicles and in postsynaptic densities in adult animals. Increased number of immuno-positive neurons were found in the middle cortical layers of adult cat when geniculate input was removed by an ipsilateral thalamic lesion performed early in life. These results show that CAM II is transiently expressed in specific populations of cortical neurons during postnatal development and that the level and distribution of the kinase is use-dependent.

409.10

ENVIRONMENTAL ALTERATION OF ONCOGENE mRNA LEVELS IN CAT VISUAL CORTEX.

M.A.McCormack, K.M.Rosen, L.Villa-Komaroff, and G.D.Mower. Neurology Research, Children's Hospital, Harvard Medical School, Boston, MA 02115.

Several aspects of postnatal visual cortical development suggest environmentally induced changes in gene expression. Specifically, dark rearing prolongs the critical period and brief (2 day) visual exposure in dark reared animals promotes rapid physiological changes. Oncogenes provide a convenient window for studying changes in signalling pathways within the cell. The present study begins analysis of the effects of dark rearing and exposure to light on oncogene mRNA levels as assayed by northern blots. We compared normal and dark reared cats at 5 and 20 weeks of age, and dark reared cats given 2 days of visual experience at 5 weeks.

To date we have examined the expression of **c-fos**, **c-myc**, and **egr-1**. Levels of **c-fos** mRNA were decreased 40% by dark rearing at both ages. **c-myc** mRNA levels were elevated by dark rearing at both 5 (90%) and 20 (30%) weeks. Lesser effects were seen with **egr-1**, which was decreased 25% by dark rearing at both ages. Brief visual exposure in dark reared animals caused additional decreases in **c-fos** and **egr-1** mRNA levels of 40%, whereas **c-myc** levels were unchanged. These environmentally induced alterations are consistent with a possible role of proto-oncoproteins in visual cortical plasticity. Analysis of additional cortical structures is underway.

409.11

PHYSIOLOGICAL ACTIVITY REGULATES zif/268 IN THE VISUAL CORTEX.

P.F. Worley, B. Christy*, Y. Nakabeppu*, and J.M. Baraban. Dept. of Neuroscience and Depts. of Molecular Biology and Genetics, Johns Hopkins Univ. School of Medicine, Baltimore, MD 21205.

In recent studies, we demonstrated synaptic activation of the transcription factor gene, zif/268, in dentate granule cells following brief tetanic stimulation of perforant-path afferents leading to long-term potentiation. To address whether zif/268 is activated by physiological activity, we have examined the regulation of zif/268 in the visual cortex. In control animals, zif/268 mRNA is present at relatively high levels in layers II-IV and VI of the visual cortex. Moreover, immunostaining is prominent in nuclei of subpopulations of neurons in these layers. Visual deprivation studies indicate that these basal levels of zif/268 mRNA and protein in visual cortex are driven by visual stimuli. Monocular injection of tetrodotoxin rapidly and selectively reduces levels of zif/268 mRNA and immunostaining in rodent primary visual cortex receiving predominant inputs from the treated but not control eye. Additionally, dark adaptation (4d) results in reversible reductions of zif/268 mRNA in visual cortex. These findings suggest that physiological synaptic activity is sufficient to elicit a robust immediate early gene response in neocortex. Accordingly, Zif/268 may help orchestrate genomic responses underlying the plasticity of mature cortical neurons to altered patterns of sensory stimulation.

409.12

VISUAL EXPERIENCE REGULATES SPECIFIC SYNAPTIC MOLECULAR COMPONENTS IN THE DEVELOPING RAT STRIATE CORTEX.

Aniek A. Schoups * and Ira B. Black, Division of Developmental Neurology, Cornell Medical Center, NY 10021, and UMDNJ/Robert Wood Johnson Medical School, Piscataway, NJ.

To study potential modulation of synaptic molecular structure by experience, the major postsynaptic density protein (mPSDp) in rat striate cortex was measured during normal development and after a period of visual deprivation. A purified synaptosomal membrane (SM) fraction from Long-Evans hooded rats was isolated, and mPSDp (separated by SDS-PAGE) was quantitated using ^{125}I-calmodulin. During normal development, mPSDp matures differently from other molecular synaptic components: whereas total SM protein increased 32-fold between days 5 and 60, the mPSDp increased 455-fold. Rats reared in complete darkness for two weeks following birth exhibited 60% of total SM protein found under normal rearing conditions (12hr light/dark cycle). Visual deprivation during a critical period thus interfered with normal development. Moreover, striate cortex mPSDp was selectively affected: following dark-rearing, striate cortex mPSDp was only 34% of normal. In contrast, light-deprivation did not alter mPSDp in parietal or prefrontal cortices, indicating topographical specificity of the synaptic response. Adult striate cortical mPSDp, on the other hand, was not affected by two weeks of dark exposure. We conclude that visual experience during development selectively modified specific components of the postsynaptic membrane structure. Since the mPSDp (MW= 51kDa) is an autophosphorylating subunit of a Ca^{2+}/calmodulin kinase, these data raise the possibility that this kinase plays a role in neural plasticity.

409.13

THE DEVELOPMENT AND DISTRIBUTION OF BETA ADRENERGIC RECEPTORS IN KITTEN VISUAL CORTEX: IMMUNOCYTOCHEMICAL AND AUTORADIOGRAPHIC STUDIES.

Y.L. Liu, W.G. Jia, A.D. Strossberg*, M.S. Cynader. Department of Ophthalmology, University of British Columbia, Vancouver, B.C. Canada. * Laboratory of Molecular Biology of Receptors, Institut Pasteur, Paris, France.

The developmental localization of beta adrenergic receptors has been studied in kitten visual cortex using immunocytochemistry and autoradiography. Using a monoclonal antibody, which recognizes beta receptors and a beta-specific ligand (I-125 CYP, with 5-HT competition) we have found specific populations of neurons that express beta receptors in the cortex. In adult animals, the receptors are concentrated in the superficial and deep cortical layers (layers 1, 2, 5 and 6). In neonatal kittens, fewer receptors are present, but they appear to be concentrated in the deep cortical layers and white matter. Receptor numbers increase after birth and by 14 days of age, the laminar distribution approaches that of the adult animals, with further thinning out of labelled cells in layer 4, between 14 and 120 days of age. Most immunostained neurons were stellate cells, although a few pyramidal cells were stained in layers 3 and 5. This pattern of distribution did not appear to be input dependent as it was not affected either by removal of thalamic input early in life, or by early interruption of adrenergic input.

409.14

THE DEVELOPMENT OF MK-801 BINDING SITES IN CAT VISUAL CORTEX.

B. Gordon, D. Parkinson and N. Daw. Dept. Cell Biology and Physiology, Washington Univ. Sch. Med., St. Louis, MO 63110.

Two facts led us to speculate that the density of binding sites associated with the N-methyl-D-aspartate (NMDA) receptor might vary with age in the cat visual cortex. First, the NMDA receptor has been implicated in neural plasticity. Second, plasticity in cat visual cortex varies with age. To test this notion we measured binding of the NMDA antagonist, MK-801, in homogenates of visual cortex membranes from cats aged 7 days (d), 21 d, 43 d, 83 d, 7-8 mos and over 2 years. Binding was measured in the presence of 100 μM glutamate, 30 μM glycine and 30 μM spermidine after 3 hours incubation at which time equilibrium had been reached.

Binding was maximal at 43 and 83 days, decreasing in both younger and older animals. Saturation analysis showed that the variation in binding with age resulted from variation in B_{max}.

Addition of glutamate, glycine and spermidine caused a greater percentage increase in binding in adult animals than in 43 day old animals. This may indicate that NMDA channels have more frequent spontaneous openings in 43 day old animals than in adults.

409.15

SUBPLATE-1: A MOLECULAR MARKER FOR EXCITATORY NEURONS IN SUBPLATE ZONE OF DEVELOPING CAT CORTEX?

P.Wahle, J.Lübke*, J.R.Naegele[1], K.Albus *MPI Biophysik.Chemie, 3400 Göttingen, FRG and [1]Dept. Ophthalmol. and Visual Science, Yale University School of Medicine, New Haven, CT 06510, USA*

A transiently expressed antigen in the cortical subplate zone of kittens was detected with a monoclonal antibody called SUBPLATE-1 (Soc. Neurosci. Abstr.15,1 '89). On Western blots of PD12 kitten cortex homogenates, a single band of 60 kD is stained. Phosphatase does not alter the staining, suggesting the epitope does not involve a phosphorylation site. SUBPLATE-1 immunoreactivity (ir) is expressed in inverted spiny pyramids, as shown by combined intracellular injection of Lucifer Yellow and immunocytochemistry. The neurons send a major dendrite toward the white matter and several minor dendrites toward the gray matter. Axons typically arise from the major dendrite and descend into the white matter forming recurrent collaterals. This morphological type is also labeled when DiI is implanted into thalamus or supragranular cortical layers of young kittens. It suggests that the SUBPLATE-1 antigen may be expressed in transient neurons of pyramidal morphology with long-range axonal projections. This concurs with double - immunofluorescence studies demonstrating that the majority of SUBPLATE-1 ir cells are neither GABAergic nor peptidergic. We suggested a glutamatergic nature, because many white matter neurons of inverted pyramidal shape display glutamate ir. Curiously, few, if any of the SUBPLATE-1 neurons are glutamatergic. It is possible that SUBPLATE-1 ir neurons contain aspartate or a neurotransmitter not yet identified in the subplate zone of cat cortex.

409.16

NEUROPEPTIDE-Y IMMUNOREACTIVITY (NPY-IR) AND SOMATOSTATIN-IMMUNOREACTIVITY (SOM-IR) FOLLOW DIFFERENT DEVELOPMENTAL PATTERNS IN CAT VISUAL CORTEX. Dale Hogan and Nancy E.J. Berman, Department of Anatomy and Cell Biology, Univ. of Kansas Medical Center, Kansas City KS, 66103.

We examined the numbers and location of NPY-ir and SOM-ir cells in postnatal kittens and adult cats to determine whether the expression of these peptides differs developmentally across visual cortical areas. The total number of NPY-ir neurons per section increases postnatally in all areas examined. Adult visual cortex has three times as many cells as the newborn. The density of cells remains the same throughout development, the increase in number being directly correlated to the increasing brain size. The increase in NPY-ir cell numbers is accompanied by an increase in NPY-ir axons. Developmentally, medial areas are invaded by immunoreactive processes first, with the lateral areas lagging about a week behind. As the axons grow into the cortex, they follow a radial pattern, growing straight up through the cortical plate and branching first in layer I. In the adult, dense fibers crisscross the whole extent of the cortex in secondary visual areas. In areas 17 and 18, stained processes avoid layer IV.

By contrast, Som-ir cell numbers decrease developmentally, and there is a clear difference across the cortical areas studied. There is no evidence of exuberance in areas 17, 18 and 19 in the one week old kitten. The number of immuno-reactive cells increases from medial to lateral and the lateral bank of lateral suprasylvian sulcus has 3 - 4 times as many cells per section as areas 17 and 18. By three weeks of age, this lateral exuberance has disappeared, and the distribution of Som-ir cells has reached the adult pattern. These results implicate somatostatin as a possible factor in guidance of axons into secondary visual areas, but do not support such a role for neuropeptide-Y.(Supported by MH38399, BNS881997, and RCD8954894)

409.17

SEROTONIN RECEPTORS EXHIBIT A TRANSIENT COLUMNAR AND LAMINAR DISTRIBUTION IN DEVELOPING CAT VISUAL CORTEX. Richard Dyck and Max Cynader, Dept. of Ophthalmology, University of British Columbia, Vancouver, British Columbia, Canada, V5Z 3N9.

The distributions of many neurotransmitter/neuromodulator receptors in the cat visual cortex show an age-related laminar specificity. None, however, have yet proven crucial in establishing the intrinsic columnar organization of the cortex during the critical period for cortical plasticity. Recent studies indicate that serotonergic (5HT) neurons may play an important role in development by affecting neuronal outgrowth and synaptic plasticity. In this study we used in vitro autoradiographic methods to assess the developmental distribution of 5HT receptors.

Six sets of adjacent sections through the visual cortex of cats ranging in age from birth (postnatal day 0; P0) through adulthood (>P150) were incubated in buffer containing either [^{3}H]-5HT, [^{3}H]-8-OHDPAT, [^{125}I]-CYP, [^{3}H]-mesulergine, [^{3}H]-ketanserin or [^{3}H]-quipazine to assess the distribution of 5HT1, 5HT1a, 5HT1b, 5HT1c, 5HT2, and 5HT3 receptors, respectively. Some sections were also incubated in the presence of μM concentrations of the appropriate competitor to ascertain binding specificity.

The distribution of each 5HT receptor subtype presented a distinct and unique developmental pattern of expression. While 5HT1, 1a and 1b binding sites exhibited laminar changes in distribution early in development, the distribution of 5HT2 sites changed relatively late. 5HT3 sites appeared to remain homogeneous throughout development. In addition to early laminar changes in distribution, **5HT1c receptors were found to be transiently expressed in columns within lamina IV between P30 and P60.** Until now, no receptor population has been shown to participate in the process of column formation in the developing cat visual cortex. The transient columnar expression of the 5HT1c sites makes them a prime candidate for this role.

NERVE GROWTH FACTORS VII

410.1

DISTRIBUTION OF NERVE GROWTH FACTOR RECEPTORS (NGFR) IN THE RAT SPINAL CORD AS A FUNCTION OF AGE AND NGF SUPPRESSION. B.A. Urschel and C.E. Hulsebosch. Dept. of Anat. and Neurosci., Marine Biomed. Inst., Univ. of Texas Med. Br., Galveston, TX 77550.

It has been postulated that NGF binding to the low affinity-fast dissociating NGFR on Schwann cells is involved with neurite extension during development and regeneration. Since central projections of somatosensory fibers sprout into the spinal cord after neonatal administration of antibodies to NGF (ANTI-NGF), it is of interest to determine if the distribution of NGFR correlates with the occurence of sprouting. Neonatal rats were given daily injections of ANTI-NGF (3μl/gm body wt.) for a period of up to one month. Treated and untreated rats were sacrificed on postnatal days (PD) 0, 7 and one month. The NGFR distribution was determined using the monoclonal antibody 192 and standard immunohistochemical techniques. In the untreated rats, NGFR distribution on PD 0 and 7 was localized in laminae I, II, III and medial IV; fasciculus cuneatus and gracilis; tracts above and below the central canal and around motorneurons. By one month of age, distribution was localized to only laminae I and II and the tracts above and below the central canal. Data from the ANTI-NGF treated rats will be presented. We hypothesize the NGFR are involved with sprouting and synaptogenesis of somatosensory components of the spinal cord.

410.2

PHASIC INCREASES IN NERVE GROWTH FACTOR OCCUR IN DORSAL ROOT GANGLIA AFTER SCIATIC NERVE CRUSH. M.R. Wells, and J.P. Schwartz. Nerve Regeneration Laboratory , Veterans Administration, Northport, N.Y. 11787 and CNB, NINCDS, NIH, Bethesda, Md. 20892.

The nerve growth factor content of sensory ganglia was examined after nerve injury to determine its possible relationship to the metabolic response of dorsal root ganglion cells to axon injury. Male, Wistar-Furth rats were subjected to unilateral crush lesions of the sciatic nerve at the level of the sciatic notch. At 1,2,3,4,5,7,8,9,11,14, and 30 days after injury, the L5 and L6 lumbar ganglia and the C6 cervical ganglia were removed bilaterally and examined for NGF content by an enzyme-linked immunoadsorbant assay. Ganglionic NGF increased up to 500% phasically and bilaterally at days 1, 4, 7, and 8 days in the L5 and L6 ganglia combined (p=0.0002, ANOVA). The strongest effect was observed bilaterally in the L6 ganglia (p= .001). The data also suggested an increase in the NGF content of cervical ganglia at 3 days after injury. Since sciatic nerve section is known to elevate NGF in Schwann cells, perhaps mediated by interleukin-1 released from macrophages, these results suggest the possibility that Schwann cells in the ganglia may also synthesize increased amounts of NGF. Why this increase should occur phasically remains to be determined. Phasic increases in ganglionic NGF coincided temporally with specific metabolic changes observed in identically axomized neurons in sensory ganglia (Wells, Exp. Neurol. 95: 313, 1987). However, in these prior studies, the pattern of responses of the injured and uninjured sides differed. The source and localization of the NGF increase will be important in determining the role it may play in the metabolism of axotomized sensory neurons. Suported by the Veterans Administration and NIH

410.3

NERVE GROWTH FACTOR (NGF) IMMUNOREACTIVITY IS RESTRICTED TO A SUBPOPULATION OF SENSORY NEURONS
R.A. Rush, R. Williams, J. Vahaviolos, R. Ressom, C. Auffray and A. Keller. Dept. of Physiology, Flinders University, Adelaide, Australia, 5042.
We have demonstrated NGF immunoreactivity in neurons of chicken peripheral ganglia using anti-peptide antisera. In dorsal root ganglia (DRG), the antigen is visible only in small neurons, with increasing concentrations, from embryonic day 10 up to adult ages. The antigen is also present within some neurons of trigeminal and jugular ganglia, but no immuno-reactivity is detectable in neurons of the vestibulo-acoustic, nodose, petrosal, ciliary or, surprisingly, sympathetic ganglia. Neither is stain detectable in either central or peripheral nerve processes except distal to a nerve ligation. Characterization of the immuno-reactivity present in DRG was achieved following chromatographic separation of ganglion extracts; antigen being found only in fractions that eluted identically with pure NGF. Extracts of sympathetic ganglia also contained immuno-chemically identifiable NGF. We conclude that the factor exists in different compartments of reactive and non-reactive neurons, and that only some of the NGF present can be detected immunohistochemically.

410.4

AGING SYMPATHETIC NEURONS WITH ELEVATED LEVELS OF CYTOPLASMIC CALCIUM DONOT DIE BUT BECOME ATROPHIC UPON DEPRIVATION OF NERVE GROWTH FACTOR IN VITRO: CHARACTERIZATION OF NEURONAL ATROPHY.
T.Koike, S. Tanaka* and S.Masuko, Depts. of Natural Sci. and Anatomy,Saga Med. Sch.,Nabeshima,Saga 84001,Japan
Sympathetic neurons become independent of NGF for survival upon aging: trophic deprivation does not cause immediate death of these neurons. We hypothesize that this is due to elevated levels of cytoplasmic free Ca^{2+}(PNAS 86:6421,1989). Indeed, the neurons with raised levels of free cytoplasmic Ca^{2+}($\geq$220nM) became resistant to NGF deprivation and their survival tended to be independent of NGF(J.Cell.Biochem. 14F:95,1990). These neurons, however, became gradually atrophic upon withdrawal of NGF: cell shrinkage occurred slowly and neurites became thinner. No major alterations were observed in the structure of cellular organelles. This process was reversible. Thus, we can dissociate the trophic effects of NGF from neuronal survival under these conditions so that normal neurons are available for studying cellular signaling associated with atrophy and hypertrophy. Neuronal atrophy caused by NGF withdrawal appears to be partly prevented by the activation of protein kinases but not prevented by high K^+(35mM) medium.

410.5

NGF and NGF Receptor Expression in Primary Reaggregate Cultures Derived From the Embryonic Mouse Septum
J.D. Roback[1], M. Downen[1], H.J. Lee[1], J. Zucker[1], T.H. Large[2], U. Otten[3], and B.H. Wainer[1]. [1]University of Chicago, Chicago IL 60637, [2]Case Western Reserve University, Cleveland OH 44106, and [3]University of Basel, Switzerland.
In the adult central nervous system, nerve growth factor (NGF) is expressed at high levels in the hippocampus while its receptor (NGF-R) is synthesized in the septum, primarily by the cholinergic neurons which project to the hippocampus. In order to better understand the regulation of NGF and NGF-R biosynthesis, we have grown either isolated hippocampal or septal cells derived from embryonic day 15 fetuses in reaggregate culture. At this developmental stage, septal and hippocampal neurons have not yet formed contacts with one another. We previously reported that the *in situ* developmental profile of hippocampal NGF mRNA and protein expression can be partially recapitulated *in vitro* by hippocampal reaggregates (Roback, et al, Dev. Biol. 137:451, 1990). We now report that septal reaggregates express both NGF and NGF-R. NGF protein rises steadily with time in these cultures. By day 21, septal reaggregates contain an average of 40 pg NGF protein/mg total protein (ca. 50% of that seen in day 21 hippocampal reaggregates). Levels of NGF mRNA are high at day 7 (11.4 ± 4.6 arbitrary O.D. units) and day 14 (11.6 ± 2.3), but decrease by day 21 (6.5 ± 1). Levels of NGF-R mRNA rise from day 7 (4.9 ± 2.2) to day 14 (8.3 ± 3.1), and then also decrease by day 21 (2.6 ± 0.8). Hippocampal reaggregates, on the other hand, express levels of NGF mRNA approximately 50% higher than those seen in septal reaggregates, but do not express detectable NGF-R mRNA. These results demonstrate that both NGF and NGF-R are synthesized in the septum in the absence of hippocampal interactions. Moreover, NGF and NGF-R mRNA expression may be coordinatly regulated in these septal cultures. In conjunction with data from other groups, this work is consistent with a model in which septal-derived NGF provides local regulation of NGF-R expression in the septum. Supported by NIH grants 5-T32HD070009, and NS-25787.

410.6

EXPRESSION OF NERVE GROWTH FACTOR RECEPTOR LIKE IMMUNOREACTIVITY IN THE FOREBRAIN OF POSTNATAL RATS.
M. Nishizuka and Y. Arai*. Dept. of Anatomy, Juntendo Univ. Sch. Med., Tokyo, Japan.
Immunocytochemical localization of nerve growth factor (NGF) receptor in the forebrain of postnatal rats was studied using specific anti-rat NGF receptor monoclonal antibody, 192-IgG. Wistar rats were deeply anesthetized with pentobarbital and perfused transcardially with a buffered paraformaldehyde at 1-10 postnatal days. The brain sections were incubated with a supernatant of hybridoma cell culture containing 192-IgG (courtesy of Prof. H. Hatanaka) and processed for ABC method. NGF receptor like immunoreactive neurons were obtained in the septum, the nucleus of the diagonal band of Broca, the ventral pallidum, and the caudate-putamen. Some rats were injected with 2.5S mouse NGF(1-2 μg/0.1% BSA) into the hypothalamus 3 days before sacrifice. The number of immunoreactive neurons increased in the ventral pallidum of NGF infused pups. Immunoreactive cells were also obtained in the arcuate nucleus of NGF infused rats. These data provide a possible explanation for our previous results that NGF facilitated the growth of transplanted hypothalamic neurons in fetal rats.

410.7

Sites of NGF and NGFR mRNA Synthesis in the Developing Rat Embryo
Esther Wheeler, Ph.D. [1], Margaret Byers, Ph.D. [2], and Mark Bothwell Ph.D. [1]
Dept. Physiology and BioPhysics[1] Dept. of Anesthesiology and Biological Structure[2], University of Washington, Seattle, WA 98195

The temporal and spatial expression of nerve growth factor (NGF) and its receptor (NGFR) were examined by in situ hybridization and immunocytochemical analyses of rat neonates and embryos aged 12 to 22 days post coitum. In addition to the expected expression in the nervous system, transcripts for both molecules were detected in a wide variety of non-neuronal cells. In the developing skin of the maxillary pad and the developing limb, NGF was localized in the epithelial and underlying mesenchymal cells. Expression of NGFR co-localized with NGF transcripts in the mesenchymal cell layers of the skin but was absent from the adjacent developing epithelium. In developing teeth, NGF and NGFR transcripts were co-localized only in the mesenchymal cells underlying the odontoblast cells. NGF and NGFR transcripts were also observed in developing myoblasts but not in fused myotubes. By contrast, NGFR mRNA, but not NGF mRNA, was detected at high levels in the mesenchymal cells associated with developing bronchiole epithelium and the epithelial cells of the developing kidney glomeruli. Curiously, NGFR glycoprotein was localized in the vesicular compartments and on the luminal surface of at least two epithelial cell types, the intestinal brush cells and the pillar cells of the developing cochlea. These results suggest NGF and NGFR are involved in the development of a variety of non-nueronal embryonic cell types. In addition, both factor and receptor are expressed over the entire course of embryonic limb development in a pattern consistent with a role in the instructive mesenchymal/epithelial interactions that effect morphogenesis.

410.8

EXOGENOUS NGF REGULATES NGF RECEPTOR (NGFR) EXPRESSION IN DISCRETE CNS REGIONS.
M. Fusco, P. Polato*, G. Vantini, M. Bentivoglio[1], A. Leon. Fidia Research Laboratories, 35031 Abano Terme, Italy and [1]Inst. of Human Anatomy, Verona University, 37134 Verona, Italy
We recently showed that exogenous NGF can regulate in vivo the expression of NGFR mRNA and corresponding protein levels in the basal forebrain cholinergic neurons. We have here evaluated the effect of exogenous NGF on NGFR in neuronal and non-neuronal structures expressing NGFR in CNS of newborn and adult rats. NGF was administered to adult rats via mini osmotic pumps (50 μg, 1 week) and to newborn rats by repetitive injections (5 μg, every other day from postnatal day 2 to 6). NGFR was immunocytochemically assessed by using the monoclonal antibody 192 IgG. Results show that, in both newborn and adult rats, NGF administration increases NGFR immunoreactivity (NGFRI) in basal forebrain as well as in other brain structures expressing NGFR. These structures include sensory pathways, brain stem sensory nuclei and some optic-related structures. In adult animals an increased espression of NGFRI was also induced by NGF in many thalamic nuclei displaying slight NGFRI in adulthood. Enhancement of NGFRI was also observed in non-neuronal cells such as ependymal cells and tanycytes. No effect of NGF was observed in spinal motor neurons and brainstem motor nuclei shown to express NGFRI only during development. These data suggest that, within the CNS, the role of NGF is broader than traditionally thought.

410.9

SCIATIC NERVES TRANSPLANTED INTO THE CNS EXHIBIT ELEVATED NERVE GROWTH FACTOR (NGF) PROTEIN LEVELS. D. J. Messersmith, M. Fabrazzo,* I. Mocchetti, and L. F. Kromer. Dept. of Anatomy and Cell Biology and FGIN, Georgetown University, Washington, DC 20007

After bilateral fimbria/fornix aspiration lesions in female adult Sprague-Dawley rats, segments of sciatic nerve were inserted rostrally into the septum and caudally into the hippocampus. Previously, we demonstrated that at two weeks post-transplantation there was a significant increase in the number of choline acetyltransferase-positive (ChAT+) cells in the medial septum compared to animals receiving lesions only (41% vs 21% of normal). In order to examine the role of NGF in the transplant-associated elevation in ChAT+ cells, we measured NGF protein levels using a two-site immunoassay. NGF levels were measured in normal nerve segments; nerves lesioned, ligated and left *in situ* for 2 weeks; and nerve grafts 2 weeks post-transplantation. Septal NGF levels were measured in normal, lesion controls, and animals receiving transplants.

NGF levels in the transplant were significantly higher than in normal nerve segments and slightly less than in lesioned nerves *in situ* (Mean NGF pg/mg wet tissue ± SEM: 1.3 ± 0.2, 0.7 ± 0.1, 2.0 ± 0.2, respectively). Septal NGF levels in lesioned animals (0.3 ± 0.03) were lower than in normal rats (0.4 ± 0.03) or in specimens with transplants (0.4 ± 0.04). These results suggest that NGF released from the transplant may be one factor that contributes to the return to normal NGF levels in the septum and supports an increase in the number of ChAT+ septal neurons after lesions. Supported by NIH grant # NS 23522.

410.10

STIMULATION OF CARNITINE ACETYLTRANSFERASE IN PC-12 CELLS BY NGF: RELATIONSHIP TO CHOLINE ACETYLTRANSFERASE STIMULATION. P.W. Scates* and H.L. White. Div. of Pharmacology, Wellcome Research Laboratories, Research Triangle Park, N.C. 27709

When incubated with rat pheochromocytoma cells (PC-12 cells), nerve growth factor (NGF) causes neurite extension, phosphorylation of kinases, and increases in certain enzyme activities, including choline acetyltransferase (ChAT). In the present study we have determined the effects of NGF on both ChAT and carnitine acetyltransferase (CarAT). PC-12 cells were cultured in serum-free DMEM +/- NGF. Cells were periodically examined morphologically, harvested, washed, lysed, and assayed for ChAT and CarAT, using [14C]acetyl -CoA and either choline or carnitine as substrates.

Basal activity of CarAT in PC-12 cells was more than 100-fold greater that that of ChAT. Both enzymes were stimulated by NGF in parallel, in a concentration and time-dependent manner. Significant stimulation was observed by 24 hr incubation with 5 ng/ml of NGF, and both activities were at least 3-fold higher after 72 hr incubation. Additional experiments showed that 10 μM or more of acetylcarnitine stimulated ChAT activity in the absence of NGF. These observations suggest that the activities of CarAT and ChAT may be coupled and that their stimulation by NGF may favor a reversal of cholinergic deficits in animal models of neuronal degeneration.

410.11

NEUROTROPHIC EFFECT OF RECOMBINANT HUMAN NERVE GROWTH FACTOR (RHNGF) IN VITRO AND IN VIVO: COMPARISON WITH THE EFFECT OF MOUSE NGF (MNGF). M.Kakihana*, M.Iwane*, K.Nakahama* and M.Suno. Biol.Res.Labs., R&D Division, Takeda Chem.Ind., Ltd., Jusohonmachi, Yodogawa-ku, Osaka 532, Japan.

Recombinant human NGF (rhNGF) was obtained by expression of human NGF gene in CHO cells and purified to near homogeneity from the culture medium. The septal neurons were dissected from 17-day fetal rat brain and seeded on a feeder layer of astroglial cells. Both rhNGF and mouse NGF (mNGF) increased acetylcholine (ACh) content and choline acetyltransferase (CAT) activity in the septal neurons in a concentration-dependent manner; the ED50 was 0.5 ng/ml for rhNGF and 2.5 ng/ml for mNGF. Neurite promoting activity of rhNGF and mNGF at a concentration of 30 ng/ml was almost the same.

In rats with unilateral fimbria-fornix transection, cholinergic cells stained by acetylcholinesterase (AChE) histochemistry in the medial septum and vertical limb of the diagonal band were reduced to 20% of that of the contralateral nonlesioned side. Administration of rhNGF (3 or 30 μg) decrease this reduction in the number of survival cells; the survival rate was 45-55%. The effect of mNGF in this system was less potent than that of rhNGF. These results indicate that rhNGF has the same (or more potent) neurotrophic effect as mNGF in vitro and in vivo.

410.12

NERVE GROWTH FACTOR RECEPTOR EXPRESSION IN THE DEVELOPING CHICK EMBRYO. Dario Marchetti, Lanny J. Haverkamp and James L. McManaman. Department of Neurology, Houston, Texas 77030.

To investigate the presence of receptors for nerve growth factor (NGFR) in developing chick embryos, iodinated β-NGF was used as a probe in a biochemical analysis performed on chick spinal cords from embryonic day 6 (E6) to E15. Strong NGFR expression was observed as early as E6 with presence of high-affinity ($K_D \approx 10^{-11}$M) and low-affinity ($K_D \approx 10^{-9}$M) NGFR. At this stage of development, we found values of NGFR/cell equal to 4,000 and 44,000 for high- and low-affinity NGFR respectively. These values declined with marked differences in the amount of receptor sites throughout the developmental time course: by E15 no specific NGF binding sites could be detected.

Interestingly, the temporal distribution of NGFR varied according to the type of NGFR considered: high affinity NGFR virtually disappeared by E10 (8% present compared to E6 value) while at the same developmental stage we were able to detect 73% of low-affinity NGFR.

The transient expression of the two NGFR and their differential distribution at the time of motoneuron synapse formation and cell death may shed light on NGF involvement in the development of non-classical targets for NGF action. Experiments to warrant a detailed analysis of NGF role in neuromuscular differentiation are in progress.

410.13

NEUROBIOLOGICAL EFFECTS OF COLCHICINE ADMINISTRATION INTO THE HIPPOCAMPUS: MODULATION BY NGF. **S. Barone Jr., M.J. Bonner, P. Tandon, H.A. Tilson. LMIN/NIEHS, Research Triangle Park, NC 27709.**

This study examines NGF modulation of signal transduction in the hippocampus (HPC) following neurotoxic lesions with colchicine (COL). Male Fischer-344 rats received COL (2.5 μg/site) or vehicle into the dorsal HPC. Immediately after COL treatment, chronic cannula were implanted unilaterally in the ventricle and modified Alzet mini-osmotic pumps (0.25 μl/hr) with β-NGF [10 ng/μl in ACSF] or cytochrome C [20 ng/μl in ACSF] were attached. One wk post-lesion animals were tested in activity chambers for 60 min. NGF treatment reduced COL-induced hyperactivity. Rats were sacrificed at 3 or 12 wks post-lesion for neurochemical or morphological analysis. Agonist-stimulated phosphoinositide (PI) turnover was measured after [^{3}H]-inositol was incorporated into HPC slices. Various treatments did not alter carbachol (CARB)-induced PI turnover at 3 wks. However, NE-induced PI turnover was increased in COL/ACSF and ACSF/NGF. At 12 wks, NE and CARB stimulation of PI turnover was increased in COL-lesioned rats. This lesion effect was blocked when rats received NGF. Sections were stained for AChE or immunocytochemically (ICC) for ChAT. COL treatment caused an increase in AChE staining in the CA3 of HPC but NGF treatment had no effect.

410.14

NATURALLY OCCURING ANTIBODIES AGAINST NERVE GROWTH FACTOR IN SERA FROM RABBITS AND HUMANS. E.Dicou[1], V.Lambropoulou[2]* and P.Markoulatos[2]*. [1]INSERM U 298 Centre Hospitalier Régional 49033 Angers France; [2]Institut Pasteur, Athens Greece.

Sera from unimmunized rabbits and from humans and Herpes simplex virus (HSV)-infected human patients were analysed for the presence of antibodies to nerve growth factor (NGF). Screening of sera was done by enzyme immunoassay using purified mouse NGF for coating of microplates. NGF antibody activity was detected with a variable titer in both rabbit and human sera. Student's t test indicated significant differences ($P < 0.02$) in NGF antibody activity between sera of HSV-infected patients ($n = 50$) as compared to controls ($n = 38$). Sera containing elevated NGF antibody activity were passed through columns of NGF immobilized to CNBr-activated Sepharose. The purified immunoglobulins immuno-precipitated mouse I^{125}-NGF as detected in SDS/polyacrylamide gels, and they stained by immunohistochemical techniques the granular convoluted tubules of the mouse submaxillary gland, the site of synthesis of this factor. Thus by three different experimental criteria we demonstrate the presence of natural antibodies to NGF in rabbit and human sera.

410.15

MOLECULAR CLONING OF PC3, A NOVEL EARLY-INDUCIBLE GENE BY NERVE GROWTH FACTOR IN PC12 CELLS. Felice Tirone, Roberta Possenti and Andrew Bradbury*, Department of Neurobiology, Consiglio Nazionale delle Ricerche, Rome, Italy

We have recently isolated four genes - PC1,2,3 and 4 - whose transcription is induced very early by nerve growth factor (NGF) in a tumour cell line of rat chromaffin cells, PC12 (Tirone, F., and Shooter, E.M. Soc.Neurosci.Abstr., 13(1):551,1987). Chromaffin cells, which are neural crest derivatives, differentiate into sympathetic neurons in presence of NGF, and the induction of these early genes is therefore associated with the commitment to the neuronal phenotype. One of these genes - PC4 - had been shown to be related to the rat γ interferon protein (Tirone, F., and Shooter, E.M. Proc.Natl.Acad.Sci.U.S.A.86:2088, 1989). We have now obtained the full sequence of the clone PC3 , which in fact was initially missing about 200 nucleotides in the 5' region, according to Northern and sequence analysis. The missing fragment had been obtained by primer extension and subsequent PCR amplification, using the method described by Frohman, M.A., et al. Proc.Natl.Acad.Sci. U.S.A.,85:8998, 1988. The complete cDNA clone is 2516 nucleotides long and presents two longer open reading frames (ORF), 420 (nt 64-483) and 374 nucleotides long (nt 1832-2205). The analysis of codon frequencies points to the first ORF as the sequence encoding the protein, with a predicted molecular weight of 15670 Da. A polyadenylylation signal is present at nucleotide 2294. Comparison of the nucleotide and predicted protein sequence with those present in the databases indicates that PC3 is a new sequence: we are now checking for the presence of specific consensus sites. PC3 RNA levels, which are induced by NGF in PC12 cells about 35 times within one hour and return to the basal level within four hr, are superinduced in the presence of NGF and cycloheximide: after 2 hr the RNA level is increased more than 100 times. PC3 is therefore induced by NGF without new synthesis of intervening proteins after the receptor activation. PC3 is induced as well by EGF in PC12 cells but weakly (few times) and is transiently expressed in the neural tube suggesting its participation in proliferative or differentiative events.

410.16

CORRELATION OF NERVE GROWTH FACTOR RECEPTOR DISTRIBUTION AND DIFFUSIBILITY WITH RESPONSIVENESS AND LIGAND-BINDING AFFINITY. A.H. Ross, G. Venkatakrishnan, C.A. McKinnon and D.E. Wolf. Worcester Foundation for Experimental Biology, 222 Maple Avenue, Shrewsbury, MA 01545

Using fluorescence microscopy and fluorescence recovery after photobleaching (FRAP), we have analyzed the distribution and diffusibility of nerve growth factor receptor (NGFr) on a series of cell lines. We have found: (1) cells expressing high-affinity responsive NGFr's display clustered NGRr's even in the absence of ligand. In contrast, nonresponsive cell lines are diffusely distributed. (2) Receptors on responsive cell lines are largely nondiffusing. Most receptors on nonresponsive cell lines are free to diffuse. (3) NGR does not greatly alter the distribution or diffusive properties of the NGRr on nonresponsive or responsive cell lines. We, thus, report a clear correlation between NGRr physical properties and biological responsiveness and suggest that clustering of NGFr prior to ligand binding is required for biological responsiveness.

410.17

THE ROLE OF CYTOSKELETAL ORGANELLES IN REGULATING THE SURFACE DISTRIBUTION OF NERVE GROWTH FACTOR RECEPTORS. P.E. Spoerri and F.J. Roisen. Department of Anatomical Sciences and Neurobiology, University of Louisville, School of Medicine, Louisville, Kentucky 40292.

The distribution of the Nerve Growth Factor (NGF)-receptor may be dependent on the underlying cytoskeleton. We employed immunocytochemistry to examine the topographical distribution of NGF-receptors on PC-12 cells at various stages of neuritogenic development. Using high resolution TEM, mAb to NGF-receptor labelled with colloidal gold was seen distinctly aligned along perikaryal and neuritic surfaces. Occasional, short, non-random arrangements suggested that endogenous molecules were bound to underlying cytoskeletal components. To determine the nature of these structural attachments, we applied cytoskeletal altering agents prior to immunolocalization of the NGF-receptor. Conditions which limit microfilament formation (cytochalasin D, 2 μg/ml) dramatically altered the distribution of the NGF-receptor along the perikaryal and neuritic surfaces. This effect was less pronounced under conditions which limit microtubule formation (Colcemid, 0.05 μg/ml). Furthermore, the relationship of these alignments to microtubules was examined with taxol (1 μM, microtubule stabilization and promotion). Studies in progress probe the role of microtubules and microfilaments in NGF-receptor distribution by applying unique neuritogenic signals known to affect neuritic cytoskeletal organization and examining the characteristic patterns of NGF-receptor produced by these signals. NGF-receptors appear to be primarily associated with cytochalasin-sensitive microfilaments which regulate their distribution on PC-12 cell surfaces. Supported by NIH grant NS24524.

410.18

RAS METABOLISM AND POST-TRANSLATIONAL MODIFICATION IN PC12 CELLS: EVIDENCE FOR A *RAS*-INDEPENDENT PATHWAY FOR NGF RESPONSES. M.-S. Qiu, A.F. Pitts, T.R. Winters* and S.H. Green. Dept. of Biology, Univ. of Iowa, Iowa City, IA 52242

We have used the drug compactin to prevent activation of *ras* protoöncogene protein in PC12 cells in order to determine whether *ras* is necessary for responses to NGF. Compactin inhibits synthesis of mevalonate, the precursor to the polyisoprene moiety that is covalently linked to *ras*. We assessed *ras* expression, turnover, post-translational modification and subcellular localization by immunoprecipitation of *ras* metabolically labeled with ^{35}S-methionine or ^{3}H-mevalonate. Treatment of PC12 cells with compactin results in a complete inhibition of *ras* post-translational modification: polyisoprenylation and proteolytic cleavage of the carboxyl terminal. Translocation of *ras* to the membrane, required for *ras* activity, is also completely inhibited by compactin. We find that *ras* has a half-life of ≈24 h in PC12 cells and a 5 d treatment is required to chase previously synthesized and isoprenylated *ras* from the cell membrane. Incidentally, we find that compactin inhibition of *ras* function or membrane localization results in a marked increase in *ras* expression. Compactin also inhibits responses of PC12 cells to transforming *ras* oncogene expressed transiently from a viral LTR promoter following transfection into the cells or expressed in a stably transfected PC12 subline from a steroid-inducible MMTV promoter. Under these conditions, in which *ras* activation is prevented, we assayed NGF responses and found that NGF-dependent cell survival, phosphorylation of tyrosine hydroxylase and induction of early gene transcription are not inhibited by compactin treatment. Compactin does inhibit NGF-induced cell surface ruffling but this effect is nonspecific: EGF-induced ruffling is similarly inhibited by compactin. Thus, *ras* function appears not to be necessary for responses to NGF. These observations, taken together with those of other laboratories, suggest that NGF signal transduction involves multiple parallel pathways with *ras* not necessarily involved in all of them.

NERVE GROWTH FACTORS VIII

411.1

INDUCTION OF MORPHOLOGICAL AND PHYSIOLOGICAL DIFFERENTIATION OF N1E-115 CELLS IN SERUM FREE MEDIUM. P. Cobbett, C. Cosgrove*, & I. Tien*. Dept. Pharmacol./Toxicol. and Neuroscience Program, Michigan State Univ, East Lansing, MI 48824-1317.

Murine neuroblastoma N1E-115 cells proliferate and are non differentiated when maintained in medium containing 10% newborn calf serum (NCS). Differentiation may be induced by addition of 2% dimethylsulfoxide to the medium (Kimhi et al., PNAS 73, 462, 1976). We have examined the effects of maintaining N1E-115 cells in serum-depleted media on cell growth, process outgrowth and membrane excitability. Media containing 1% or 2% NCS inhibited growth compared to cells grown in 10%NCS medium but did not induce morphological differentiation. Cells in 0% NCS medium for more than 3 days had significantly reduced population growth and increased process outgrowth. By day 5, >30% cells had processes longer than the somatic diameter whereas <5% of cells in 10% NCS medium had processes. Cells in 10% NCS medium or 0% NCS medium for <4 days had similar resting potentials but cells in 0% NCS medium had a significantly increased input conductance: 10% NCS 1.0± 0.1 nS, 0% NCS 2.0± 0.3 nS. Non-differentiated cells were essentially inexcitable, but cells in 0% NCS became more excitable with time. The action potential amplitudes were: 4 days 39.3± 3.8 mV; 8 days 53.6± 3.2 mV; and 13 days 57.4± 5.3 mV. Only cells maintained in 0% NCS medium for 8 or 13 days generated repetitive current-evoked or spontaneous action potentials: these had TTX-sensitive and Ca^{2+}-dependent components. Serum removal clearly differentiated N1E-115 cells morphologically and physiologically indicating the presence of one or more serum factors which directly or indirectly inhibits differentiation. Further, morphological and physiological differentiation were temporally different suggesting that the two processes may be controlled by separate mechanisms. (Supported by PMAF)

411.2

EXPRESSION OF NEURONAL PHENOTYPE IN BOVINE ADRENAL MEDULLARY CHROMAFFIN CELLS S. Sleight and M.D. Browning. Department of Pharmacology, University of Colorado HSC, Denver, CO 80262.

Chromaffin cells of the adrenal medulla have long been used as a model for neurosecretion. Recently, the potential therapeutic application of human chromaffin cell transplants in Parkinson's disease has been described. In both instances, expression of neuronal phenotype by chromaffin cells would be particularly valuable. Unsicker and colleagues have demonstrated that primary cultures of bovine adrenal medullary chromaffin cell cultures exhibit modest neurite outgrowth when plated on collagen. We have monitored the expression of 2 characteristics of neuronal phenotype in such cultures. We confirmed Unsickers' work in highly purified chromaffin cell (95%) cultures, observing the flattening of the cells and expression of short neurites (length equivalent to 1 cell diameter). However, in partially purified chromaffin cell preparations (60-70% chromaffin cells, 20-30 % cells resembling fibroblasts), an extensive network of neurite extension was observed. Essentially 100% of the chromaffin cells exhibited neurite extension of distances 4-5x the diameter of the cell body. Moreover, transfer of media from these cultures to the highly purified cultures resulted in extensive neurite outgrowth in these cultures. Secretion of catecholamines was also monitored in these cultures. Highly purified cultures exhibited essentially invariant secretion with time in culture. However, in the partially purified cultures of chromaffin cells secretion increased in parallel with the extensive neuritic growth. Identification of the factor(s) responsible for the expression of neuronal phenotype in these cultures is currently under investigation. Particular attention is being given to the contribution of the various fibroblast derived growth factors. Supported by PHS grant DK40483 and NS26377 to MDB.

411.3

MOLECULAR CLONING OF AN NGF-INDUCIBLE KINASE: THE DEFINITION OF A NEW FAMILY OF KINASES WHICH MEDIATE RESPONSES TO EXTRACELLULAR SIGNALS. G.D. Yancopoulos, T. G. Boulton* and M. H. Cobb.* Regeneron Pharmaceuticals, Inc. 777 Old Saw Mill River Road, Tarrytown, NY 10591.

A serine/threonine kinase (MAP-2 kinase) which can phosphorylate microtubule-associated protein (MAP-2) *in vitro* apparently acts as an intermediate in phosphorylation cascades induced by a variety of growth factors such as insulin and EGF. Evidence suggests that this MAP-2 kinase is rapidly activated following tyrosine phosphorylation by receptor or non-receptor tyrosine kinases. Activation of MAP-2 kinase is also one of the earliest detectable cellular events induced in PC12 cells by nerve growth factor (NGF). To facilitate further studies of MAP-2 kinase, we have molecularly cloned and sequenced MAP-2 kinase from a rat brain cDNA library; our cloning strategy led to the identification of additional kinases, also expressed in the rat brain, which are highly related to MAP-2 kinase. These kinases form a new family of mammalian kinases which may play crucial roles in the response to mitogens and differentiation factors. Interestingly, these mammalian kinases are most closely related to two protein kinases, recently cloned from yeast, which are thought to play antagonistic roles in pheromone-induced cell-cycle arrest.

411.4

NEUROTROPHIN-3: MOLECULAR CLONING AND COMPARISON OF DEVELOPMENTAL EXPRESSION TO NGF AND BDNF. P.C. Maisonpierre, L. Belluscio*, R.M. Lindsay and G.D. Yancopoulos. Regeneron Pharmaceuticals, Inc. 777 Old Saw Mill River Road, Tarrytown, NY 10591.

The restricted neuronal specificity of NGF has long suggested that other neurotrophic factors exist which act on neuronal populations that are refractory to NGF. Recent cloning of brain-derived neurotrophic factor (BDNF) has revealed that it shares close structural similarity to NGF. Using a PCR-based approach that exploited this similarity, we have identified a novel member of the NGF-BDNF gene family, termed neurotrophin-3 (NT-3). Like NGF and BDNF, NT-3 is synthesized as a precursor protein that is cleaved to form a mature protein that has 57% and 58% amino acid sequence identity with NGF and BDNF, respectively. Recombinant NT-3 protein displays distinct neuronal specificity, compared to NGF and BDNF, when assayed on explant and dissociated cultures of PNS and CNS neurons. In contrast to the limited peripheral distribution of BDNF mRNA, NT-3 and NGF transcripts are widely expressed among adult rat peripheral tissues. In the adult CNS, all three factors are expressed at highest levels in the hippocampus; NT-3 and BDNF share other similarities in their CNS distribution. Interestingly, particular cell populations of fetal and newborn rat express NT-3 at dramatically higher levels than NGF and BDNF. The specific detection of localized, abundant NT-3 transcripts early in development points to populations of neuroblasts/neurons that may be dependent on NT-3 for their proliferation, differentiation and/or survival.

411.5

MOLECULAR CLONING AND EXPRESSION OF THE HUMAN CILIARY NEUROTROPHIC FACTOR (CNTF) GENE. P.Masiakowski, H. Liu*, R.M. Lindsay, M.E. Furth and N. Panayotatos. Regeneron Pharmaceuticals, Inc. 777 Old Saw Mill River Road, Tarrytown, NY 10591.

The diverse effects of CNTF on neuronal and non-neuronal cells, observed *in vitro*, suggest important roles for this factor in the development and regeneration of the nervous system. To explore its potential, we have cloned, sequenced, and expressed in *E. coli* the human CNTF gene. The gene codes for a 200 amino acid protein, which shares about 80% identity with its rat and rabbit homologues, and also lacks an apparent secretion signal sequence. In the optimal bacterial expression system, recombinant human CNTF constituted approximately 30% of total cellular protein. The protein has been purified to apparent homogeneity. In dissociated, neuron-enriched cultures of E8 chick embryo ciliary ganglia, neuronal survival was detectable at 100 pg/ml; maximal activity was observed at 1-2 ng/ml, with EC_{50}=500 pg/ml CNTF.

411.6

STRUCTURE-FUNCTION STUDIES OF NGF, BDNF AND NT-3. N. Y. Ip, R. M. Lindsay, M.E. Furth, S.M. Bianco*, D. R. Gies*, S. P. Squinto and G. D. Yancopoulos. Regeneron Pharmaceuticals, Inc. 777 Old Saw Mill River Road, Tarrytown, NY 10591.

Molecular cloning and sequence analyses of the nerve growth factor (NGF)-related neurotrophic factors, brain-derived neurotrophic factor (BDNF) and the recently identified neurotrophin-3 (NT-3), have revealed striking conservation among a variety of species. All of these factors appear to be synthesized as larger precursors that are cleaved to release the mature proteins which can support the survival of distinct neuronal populations. Analyses of the precursor sequences reveal homologous regions which may be important for precursor function. Comparisons between the mature protein sequences highlights regions that may be important in determining the distinct neuronal specificities of these factors. We have functionally expressed a series of genetically-modified NGF, BDNF and NT-3 molecules. The biological activities of these modified neurotrophic molecules provide unique insights into the relationship between their structure and function.

411.7

EFFECTS OF BRAIN DERIVED NEUROTROPHIC FACTOR (BDNF) ON RAT SEPTAL CHOLINERGIC NEURONS IN CULTURE

R.F. Alderson, A.L. Alterman*, Y.-A. Barde[1], and R.M. Lindsay.
Neurobiology Section, Regeneron Pharmaceuticals Inc., 777 Old Saw Mill River Rd., Tarrytown, NY 10591. 1. Max-Planck Inst. for Psychiatry, Dept. of Neurochemistry, D-8033 Martinsried Munich, Fed. Rep. of Germany.

BDNF and NGF share extensive sequence homology (Leibrock et. al., Nature, 1989). When tested for cholinotrophic activity on E17 rat septal cholinergic neurons in culture, BDNF produced a dose dependent increase in CAT activity. The maximum response occurred with 50ng/ml of BDNF and produced a 2.2-fold increase in enzyme activity. Time course studies demonstrated a linear rise in CAT activity during the first 3 to 6 days *in vitro* at which time the change plateaued. The extent of the increase in CAT activity produced by BDNF was equal in glia-containing or neuron-enriched cultures suggesting that BDNF is not acting through glia. The accumulation of choline via a Na+-dependent choline-uptake mechanism was increased 3.8-fold following treatment with 50 ng/ml of BDNF. The number of acetylcholinesterase positive cells per well was also increased, 2.5-fold, as compared to the non-treated controls following a 12 day treatment with BDNF.

411.8

NEUROTROPHIN-3 (NT-3): NEURONAL SPECIFICITY IN THE PERIPHERAL NERVOUS SYSTEM OF THE CHICK.

R. M. Lindsay, P. Maisonpierre, S. Squinto and G.D. Yancopoulos. Regeneron 777 Old Saw Mill River Road,Tarrytown, N.Y. 10591.

We have recently described the molecular cloning of a novel neurotrophic factor, which by sequence comparison is clearly a member of the nerve growth factor (NGF) / brain-derived neurotrophic factor (BDNF) family and has therefore been named "neurotrophin-3 ", (NT-3). Preliminary studies with recombinant NT-3 produced in COS cells, showed that NT-3 promoted survival and neurite outgrowth from explanted and dissociated neuron-enriched cultures of E8 chick embryo sensory neurons of both the neural crest-derived dorsal root ganglion and the neural placode-derived nodose ganglion. To further define the neuronal specificity of NT-3, we have now studied the effects of NT-3 on explant and dissociated cultures of sympathetic, parasympathetic, neural crest-derived (dorsal root, medio-dorsal trigeminal, jugular) and neural placode-derived sensory ganglia (nodose, petrosal, geniculate, vestibular and ventrolateral trigeminal) from chick embryos of 4-12 days of incubation (E4-E12). Placodal sensory neurons (especially nodose and petrosal) are highly responsive to NT-3 as early as E5, while crest-derived sensory neurons are responsive from E6 onwards. Sympathetic neurons show only modest response from E7 onwards, while parasympathetic neurons are refractory to NT-3 at all stages examined. With all of the ganglia we have examined additive effects of BDNF, NGF or CNTF (ciliary neurotrophic factor) with NT-3, particularly in relationship to the relative abundance of each growth factor in the specific target tissues of sub-populations of sensory neurons. These studies will be reported.

411.9

SPECIES-SPECIFIC MONOCLONAL ANTIBODIES TO THE CARBOXYL-TERMINUS OF HUMAN CILIARY NEUROTROPHIC FACTOR (CNTF).

M. E. Furth, D. M. Morrissey*, T. A. Aldrich*, L. Uffenheimer*, A. P. Mickle*, and N. Panayotatos. Regeneron Pharmaceuticals Inc., 777 Old Saw Mill River Road, Tarrytown, NY 10591.

The human CNTF gene has been identified and cloned, and its protein product expressed at high levels in *E. coli*. Monoclonal antibodies were obtained by fusion of splenocytes from mice immunized with the purified recombinant protein to SP2/0 myeloma cells. Positive hybridomas were identified by an antibody-capture assay using CNTF adsorbed to polystyrene.

One set of eight monoclonal IgG antibodies derived from a single immunized mouse react strongly with recombinant human CNTF but not with recombinant rat CNTF in the antibody-capture assay and by Western immunoblotting. None of these antibodies binds to a truncated CNTF protein lacking the 16 carboxyl-terminal amino acids, obtained by deleting sequences downstream from a unique BamH1 restriction site in the human gene. This truncated CNTF retains potent neurotrophic activity. Thus, the antibodies recognize human-specific epitope(s) in a non-essential region at the carboxyl-terminus of CNTF. Several human neuronal tumor-derived cell lines were found to have specific binding sites for CNTF and to respond to the factor, as judged by the induction of c-*fos* mRNA. The monoclonal antibodies to the carboxyl-terminus of human CNTF can be used to detect the ligand when bound to such cells. These antibodies may therefore prove useful in the isolation and characterization of CNTF receptors.

411.10

BRAIN DERIVED NEUROTROPHIC FACTOR PROMOTES SURVIVAL OF DOPAMINERGIC NEURONS OF THE EMBRYONIC RAT MESENCEPHALON.

C. Hyman, M. Hofer*, Y-A. Barde*, and R.M. Lindsay. Regeneron Pharmaceuticals, Inc. 777 Old Saw Mill River Road, Tarrytown, NY 10591 & *Dept. Neurochemistry, Max-Planck Institute for Psychiatry, D-8033 Planegg Martinsried, FRG.

The effects of brain derived neurotrophic factor (BDNF) on the survival and maturation of dopaminergic neurons of the presumptive substantia nigra were examined *in vitro*. Neurons dissociated from the fetal rat ventral mesencephalon were cultured under serum-free conditions. Immunocytochemical staining with antibodies to tyrosine hydroxylase (TH) and uptake of ^{3}H-dopamine were used to monitor the survival and maturation of the dopaminergic neurons present in these cultures. In the absence of exogenous neurotrophic factor(s), there was a 67% loss of TH positive neurons between day three and day eight in culture; although dopamine uptake showed an initial rise during the first eight days, this also declined with time in culture. A single treatment with BDNF one day after placing the cells in culture increased the survival of TH positive neurons by 1.9- fold eight days later. If BDNF was added in multiple doses at culture days 1,2,4,6 and 8, the relative increase observed by culture day 11 was 2.7-fold. The response to brain derived neurotrophic factor was dose-dependent and specific, in that nerve growth factor (NGF) was without effect. Experiments in which the addition of BDNF was delayed for several days indicated that observed effects of BDNF were mediated by increasing cell survival and not merely by increasing expression of TH immunoreactivity. These finding may have implications for the development of a novel therapeutic approach to Parkinson's disease.

411.11

A MUSCLE-DERIVED FACTOR PROMOTES BOTH SURVIVAL AND GROWTH BUT NOT CHOLINE ACETYLTRANSFERASE ACTIVITY IN CHICKEN SPINAL MOTOR NEURONS.

T.H. Oh, S.J. Jeong, S.L. Hobbs and G.J. Markelonis. Dept Anatomy, Univ Maryland Sch Med, Baltimore, MD, 21201.

Developing embryonic neurons require a target-derived trophic factor for survival. A protein distinct from other known neurite-inducing proteins was substantially purified from muscle tissue (Oh et al., Dev. Biol., 127:88, 1988). Purified motor neurons prepared from 6-day-old chicken embryos were used in assessing the trophic activity of this protein. Approximately 25% of the neurons initially seeded survived for 6 days in the presence of the protein. The survival rate was further enhanced by the addition of laminin. However, the specific activity of choline acetyltransferase and the level of activity of the high affinity choline uptake system were not changed after 6 days. This suggests that although this trophic protein supports motor neuron survival, it has no specific effect on the cholinergic properties of motor neurons. The protein was also found to support the survival of chicken embryonic dorsal root ganglionic neurons, sympathetic and ciliary ganglionic neurons. The survival of both DRG neurons and sympathetic neurons was increased further by the presence of NGF. The trophic protein has been purified and migrates as a doublet with MWs of 53 and 57 kDa on SDS-gel electrophoresis. Preliminary amino acid data suggest that the 53 and 57 kDa proteins are either closely related or identical. (Supported by NIH grant NS 15013 and by the Bressler Research Fund).

411.12

REGULATION OF NERVE GROWTH FACTOR SYNTHESIS BY FIBROBLAST GROWTH FACTOR IN ASTROCYTES

K. Yoshida, and F.H. Gage. DEPT. OF NEUROSCIENCES, UNIVERSITY OF CALIFORNIA-SAN DIEGO, LA JOLLA, CA 92093.

The mechanism regulating NGF synthesis in astrocytes was investigated.Astrocytes obtained from newborn rat brains were seeded on a 24-well culture plate. NGF levels were measured by two-site enzyme-immunoassay. NGF secretion by astrocytes was highest just after passage, and then gradually decreased. There was no difference in NGF secretions by astrocytes from five regions: cerebral cortex, striatum, hippocampus, septum, and cerebellum. The effects of growth factors and lymphokins were tested. Although, PDGF, IL-3, and IL-6 had no significant effect, acidic and basic FGFs, EGF, IL-1, and TNF-∂ increased NGF secretion. Among these, aFGF has the most potent effect, and the next is bFGF. IL-1, and TNF-∂ increased NGF secretion in the presence of aFGF, while bFGF and EGF did not. The peak of NGF secretion occurred 3-12 hrs after the addition of aFGF. On the other hand, increase of cell number became significant 12-48 hrs after FGF-stimulation. Although astrocytes become fibrous-shaped by FGF-stimulation, no significant morphological change was observed in the first 12 hrs. NGF secretion by astrocytes in the presence or absence of aFGF was completely inhibited by the addition of cycloheximide or actinomycin-D. FGFs also increased NFG secretion by fibroblasts derived from meninges, but not by microglia. These results indicate that FGFs activate astrocytes to produce NGF in damaged brain in consert with other growth factors and lymphokines.

411.13

BASIC FIBROBLAST GROWTH FACTOR ENHANCES THE NGF RECEPTOR PROMOTER ACTIVITY IN HUMAN NEURO-BLASTOMA CELLS; CHP100.

M. Taiji, K. Taiji*, K.L. Deverle* & M. Bothwell. Dept. of Physiology & Biophysics, Univ. of Washington, Seattle, WA98195.

Human neuroblastoma cells (CHP100) have been used to develop an *in vitro* model for sympathetic neuron differentiation. Basic fibroblast growth factor (bFGF) induces morphological changes in CHP100 cells, including neurite outgrowth and hypertrophy; in contrast, NGF does not induce any morphological changes in CHP100 cells, although immunocytochemical staining shows some endogenous expression of human NGF receptor (hNGFR). To assess the effect of NGF and bFGF on the hNGFR promoter, whose activity is known to be developmentally regulated, a 6.7kb 5'-flanking region containing hNGFR promoter was isolated from the hNGFR genomic clone and linked to the reporter gene chloramphenicol acetyltransferase (CAT). This hNGFR promoter-CAT fusion gene was transfected into CHP100 cells, and stably transformed cells expressing CAT activity were selected. The level of CAT activity in these cells was examined following treatment with bFGF or NGF. bFGF (10 ng/ml) increased the CAT activity in these transformed cells. However, NGF (100 ng/ml) did not significantly change the CAT activitiy. These results indicate that the hNGFR promoter activity in CHP100 cells is initially regulated by bFGF, but not by NGF.

411.14

DIFFERENTIAL EFFECTS OF aFGF AND bFGF ON SYMPATHETIC OUTGROWTH FROM NEONATAL MOUSE SUPERIOR CERVICAL GANGLIA IN VITRO.

M. Deschuyteneer, K.W. Roche* and J.N. Davis. Neurology Division, Dept. of Medicine; Duke University Medical Center; Durham, NC 27710.

Fibroblast growth factor appears to influence the kinetics and morphology of neuronal outgrowth in a variety of *in vitro* models. We have compared the effects of human recombinant acidic FGF (aFGF) and human recombinant basic FGF (bFGF) at various concentrations (10, 50, 100 and 500 ng/ml) on neurite outgrowth from sympathetic neurons. Superior cervical ganglia from 2-3 day old mice were cultured for several days on collagen-coated coverslips. The lengths of extending neurites and the outgrowth areas were measured using image analysis. Ganglia morphology was studied with light and scanning electron microscopy. In the absence of growth factors, the outgrowth was sparse, consisting of non-neuronal cells (NNC) and few scattered neurites. Both aFGF and bFGF stimulated the asymmetrical and heterogeneous outgrowth of numerous NNC. In addition, aFGF consistently promoted the outgrowth of neurites. They formed a fine and relatively dense network. The neurites grew on top of the non-neuronal cells to which they were closely associated. In contrast, neurite outgrowth elicited by bFGF was not consistent and was seen mostly at the highest concentrations tested (100, 500 ng/ml). The morphology of this neurite outgrowth was similar, but less dense than that observed with aFGF. Heparin was required for the aFGF effects.

Thus aFGF is more potent than bFGF in stimulating sympathetic neurite outgrowth. FGFs may have a direct effect on sympathtetic neurons or indirectly stimulate outgrowth by acting on the NNC.

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412.1

SURVIVAL OF RETINAL GANGLION CELLS "IN VITRO": EFFECT OF CONDITIONED MEDIUM FROM RETINAL CELL AGGREGATES. E. G. de Araujo* and R. Linden. Instituto de Biofisica, UFRJ, Rio de Janeiro, 21944, Brasil.

Previous studies indicated that the survival of developing ganglion cells "in vivo" depends on other retinal cells. We are now studying the degeneration of retinal ganglion cells "in vitro". Retinae of newborn rats were retrogradely labeled with horseradish peroxidase injected in the superior colliculus. One day later, the retinal cells were dissociated and cultured for 1-3 days, and the survival rates of labeled ganglion cells were evaluated. We tested the effects of culture media conditioned for 4-7 days on aggregates or explants of neonatal retinal cells. It was found that both conditioned media increased about twice the rate of survival of ganglion cells after 2-3 days in culture, when compared with control medium. The data presented here suggest that soluble molecules released by intrinsic retinal cells contribute to the control of ganglion cell survival "in vitro". (CNPq, FINEP, FAPERJ, CEPEG-UFRJ).

412.2

TROPHOMORPHISM: A UNIFYING HYPOTHESIS TO EXPLAIN NEURONAL REARRANGEMENTS. K.A. Crutcher and B.N. Saffran, Dept. of Neurosurgery, University of Cincinnati Medical Center, Cincinnati, Ohio 45267.

The results of experiments in which intraventricular infusion of NGF was found to cause remodeling of mature uninjured sympathetic axons led us to postulate that alterations in trophic support in the absence of injury may be sufficient to elicit axonal remodeling (trophomorphism) (Saffran and Crutcher, Brain Research, in press). A review of several other examples of developmental and injury-induced neuronal rearrangements also provide support for this hypothesis and suggest that a common mechanism might underlie these phenomena. We propose that the relative distribution of trophic support to different neuritic branches of the same neuron determines the survival and distribution of the neurites. This hypothesis is based on four postulates: 1) Nerve cells require a minimum amount of trophic factor for survival (trophic theory of neuronal connections), 2) Nerve cells extend neurites in order to obtain trophic support, 3) Nerve cells maximize the amount of trophic support acquired relative to the volume of cytoplasm they maintain, 4) Neuritic branches from the same nerve cell compete with each other for growth materials from the cell body (Smalheiser and Crain's "sibling neurite bias" hypothesis, J. Neurobiol., 15:517, '84). If these postulates are correct, changes in the distribution of trophic support, or injury to axonal branches that disrupt the acquisition of trophic factor by the nerve cell, result in neuronal remodeling in order to maximize trophic support to the cell body. Such a mechanism is consistent with normal developmental remodeling of axonal arbors, the retention of normally-transient branches following elimination of collaterals, and injury-induced neuronal rearrangements in maturity. (Supported by NS-17131 and AG-07691).

412.3

NETWORKS FORMED BY DORSAL ROOT GANGLION CELLS IN ORGANOTYPIC CULTURES : A COMPUTER-AIDED ANALYSIS OF HRP INTRACELLULARLY LABELED NEURONS. **M.C. Calvet, M.J. Drian* and J. Calvet*. INSERM U-336, EPHE, Institut de Biologie, 34060 Montpellier, FRANCE**

The patterns of growth, the arborizations and the neuritic pathways formed by fetal rat dorsal root ganglion (DRG) cells were compared when cultured with or without organized spinal cord explants. Intracellular iontophoretic HRP staining of the cultured DRG neurons allowed a 3-dimensional reconstruction of these individually labeled cells and a morphometric analysis of their neuritic processes. Two populations of DRG neurons were studied : 1 - Isolated DRG cells explanted at 15 to 17-day fetal rats. 2 - DRG cells with their attached transverse cross-section of whole spinal cord and explanted at 13 to 14-day fetal rats. When compared to attached neurons, the isolated DRG neurons showed longer neuritic lengths, larger neuritic field areas and more numerous branches developing in a multipolar way. The DRG neuron attached to spinal cord had significantly smaller and mostly bipolar processes with well individualized central and peripheral branches. Such preliminary results indicate that the spinal cord (and more precisely the target cells of the dorsal horn) acts as a prominent factor upon the development of the DRG neuritic networks.

(Work supported by IPSEN-BEAUFOUR)

412.4

PRODUCTION OF DIFFERENT TRUNCATED FORMS OF RECOMBINANT HUMAN NERVE GROWTH FACTOR RECEPTOR IN A BACULOVIRUS SYSTEM. V. Prabhakar, J.L. Goerke* and A. H. Ross. Worcester Foundation for Experimental Biology, 222 Maple Avenue, Shrewsbury, MA 01545.

Recombinant extracellular domain (RED) of human nerve growth factor receptor (NGF-R) is being produced in bulk quantities for biochemical characterization and X-ray crystallography using a recombinant baculovirus in Sf9 (Spodoptera frugiperda) cells in suspension culture. The RED in preliminary studies was found to be a monomer by chemical crosslinking and equilibrium centrifugation. The RED is asymmetric with a frictional coefficient of 1.7. When recombinant virus encoding RED was injected into Trichoplusia ni larvae, the RED produced (TnRED) was found to be glycosylated unlike that produced in Sf9 cells (Sf9RED). There does not seem to be any significant change in the binding pattern of TnRED with ^{125}I-NGF compared to the Sf9RED. TnRED is heat stable retaining at least 60% of the specific binding even after heating at 100°C for 15 min. This stability may result from the large number of RED disulfide bonds. Binding studies of ^{125}I-RED with NGF-responsive but NGF-R lacking neuronal cell types are in progress, in a bid to identify the NGF-R associated proteins. For the same purpose, other recombinant viruses encoding the extracellular, the transmembrane and intracellular domains are being produced.

412.5

IN VITRO ANALYSIS OF ASTROCYTE MEDIATED NEURITE BEHAVIOR IN THE HIPPOCAMPUS OF THE GRAY SHORT-TAILED OPOSSUM. R.K. Lartius and E.Uemura. Department of Veterinary Anatomy, Iowa State Univ., Ames, IA 50011.

The intimate relationship between the neuron and glial cell plays a key role in the development of the central nervous system. In this study we examined changes in neurite morphology which occurred when neurons and astrocytes isolated from the hippocampus of the Brazilian gray short-tailed opossum were co-cultured under various conditions. Astrocytes were harvested on day P19 from opossum hippocampi and grown to confluency. Conditioned medium was collected every 2 days for 10-14 days. At this time the cells were trypsinized and co-cultured with hippocampal pyramidal cells isolated on day P8-9. Control cultures of hippocampal neurons alone were simultaneously plated. Glial monolayers were prepared 1 day prior to the seeding of hippocampal neurons. As expected, it was observed that neurons grown on glial monolayers tended to be multipolar with extremely lengthy processes. Further, their long-term survival was greatly enhanced compared to control cultures. Astrocyte-conditioned media also enhanced neurite outgrowth and survival of astrocyte-free cultures. In contrast, when neurons and astrocytes were dispersed and plated at a ratio of approximately 5 to 1, neurons exhibited multipolar, bipolar, or unipolar characteristics depending on the distance between the cells and the level of neurite-neuron and neurite-astrocyte contact. If the neuron made no contact with a glial cell or it made contact but its soma was within 3 cell bodies of the astrocyte, there was a high probability that the cell would display multipolar characteristics. If the neuron was more than 3 cell bodies away and made contact with the glial cell alone, there was a high probability that the neuron would be bipolar or unipolar. Neurons which were in contact with other neurons generally remained multipolar regardless of their relationship with the astrocyte. These findings suggest that both soluble and membrane bound glial factors exert influence on neurite outgrowth during development.

412.6

CULTURED RAT ASTROCYTES EXPRESS FUNCTIONAL INTEGRINS. **N.J.Tawil and S.Carbonetto. The Centre for Neurosciences, McGill University, Montreal General Hospital Research Institute, Montreal, Canada H3G 1A4.**

We had shown earlier that Mab 3A3, which is directed against the rat α_1 integrin subunit, labelled the cell surface of rat astrocytes (Tawil et al., 1990, Biochemistry, in press). Using polyclonal antibodies against the β_1 and β_3 integrin subunits in cell attachment assays we have shown that astrocytes bind to different extracellular matrix molecules such as laminin, collagen, fibronectin and vitronectin through receptors of the integrin family. Immunoprecipitation of these integrins suggests that two heterodimers ($\alpha_1\beta_1$ and $\alpha_3\beta_1$) participate in the attachment of astrocytes to laminin, collagen and fibronectin. Astrocytes also express a heterodimer from the β_3 subclass, $\alpha_v\beta_3$, which mediates the attachment of astrocytes to vitronectin. We have also shown that Schwann cells and oligodendrocytes express β_1 and β_3 integrins. These data suggest that integrins are important for glial-matrix adhesion. We are presently looking at integrin expression and function on glia in vivo.

412.7

GANGLIOSIDE GM1 POTENTIATES THE RISE IN INTRACELLULAR FREE-Ca^{++} DUE TO K^+ DEPOLARIZATION IN PC12 CELLS. B. S. Hilbush and J. M. Levine. Dept. of Neurobiology and Behavior, SUNY at Stony Brook, Stony Brook, NY 11794.

Gangliosides are highly enriched components of the neuronal cell surface which exert neuritogenic and neurotrophic effects when added exogenously to cells in culture. These effects include the potentiation of nerve growth factor (NGF)-induced neurite outgrowth and NGF-mediated activation of protein kinases in PC12 cells. Ganglioside GM1 stimulation of a Ca^{++}- dependent protein kinase activity in K^+ depolarized or NGF treated cells (Hilbush and Levine, submitted) suggests that gangliosides may modulate signal transduction pathways dependent upon the entry of extracellular Ca^{++}. Ganglioside dependent changes in intracellular-free Ca^{++} could account for these effects. We tested this possibility by measuring the changes in $[Ca^{++}]_i$ due to K^+ depolarization in cells pretreated with GM1 ganglioside.

PC12 cells cultured on poly-L-lysine/laminin coated glass coverslips were incubated in the presence or absence of 10 μM GM1 for 2 hr followed by incubation with 1 μM Fura 2AM for 30 min. Fluorescence intensities were measured from single cells after excitation at 355 and 380nm and $[Ca^{++}]_i$ was determined by the ratio method (Grynkiewicz, et al, J. Biol. Chem. 260:3440.). Cells were depolarized by bath application of saline containing 30-60 mM KCl for 2-5 min. The rise in $[Ca^{++}]_i$ was compared between identically treated pairs of individual cells from GM1 treated and untreated dishes. GM1 treatment caused an enhanced response to K^+ depolarization in >90% of the pairs; the rise in $[Ca^{++}]_i$ due to 30 mM K^+ increased from 93 ± 20 nM in untreated cells to 205 ± 41 nM in GM1 treated cells. Brief application of GM1 (1-5 min) over a wide range of concentrations had no effect on $[Ca^{++}]_i$ by itself or when applied to cells depolarized with K^+. Thus, the exposure of PC12 cells to ganglioside GM1 for 2 hr results in a significant enhancement of the ensuing rise in $[Ca^{++}]_i$ due to K^+ depolarization. These results suggest that the neuritogenic and neurotrophic properties of exogenous gangliosides may be due to their ability to augment changes in $[Ca^{++}]_i$ resulting from normal extracellular stimuli.

412.8

DISTRIBUTION OF CALCITONIN GENE RELATED PEPTIDE (CGRP) IMMUNOREACTIVITY IN THE EMBRYONIC AND NEONATAL RODENT OLFACTORY SYSTEM H.Baker[1], M. Bailey[2] and M.T. Shipley[2] [1]Cornell Univ. Med. Coll., White Plains, NY 10605 and [2]Univ. Cincinnati Coll. Med., Cincinnati, OH, 45267.

The mechanisms by which olfactory receptor cells exert their trophic influences on development and maintenance of phenotypic expression in the olfactory bulb have not been established. A recent report (Nature, 339:701, 1989) suggested that CGRP is localized to olfactory receptor cells and regulates the expression of the dopamine phenotype in dispersed cultures of embryonic olfactory bulb. The *in vitro* findings were interpreted to suggest that CGRP is the endogenous molecule which regulates the dopamine phenotype *in vivo*. The current studies investigated whether, *in vivo*, CGRP exhibits a distribution in either adult or embryonic olfactory neurons consistent with its putative trophic role in the regulation of the dopamine phenotype. Two CGRP antisera were utilized. One, obtained from Amara and Blakely (Yale Univ.), was the same as that used in the previous *in vitro* studies. The peptide was not found in olfactory receptor cells of rat and mouse embryos examined both prior to and after the appearance of tyrosine hydroxylase, the first enzyme in dopamine biosynthesis. In the same tissue sections, CGRP immunoreactivity was found in other CNS and PNS neurons, such as spinal cord and trigeminal ganglion, respectively. In addition, varicose fibers, presumably of trigeminal origin, were present in the lamina propria of late embryonic and neonatal olfactory epithelia. In adult rat, a small number of receptor cells and varicose fibers exhibited CGRP-like immunoreactivity. These data demonstrate that, although CGRP may influence dopamine expression in culture, *in vivo* this peptide does not display the appropriate temporal or spatial distribution necessary for trophic regulation of the phenotype in either developing or adult rodents. Supported by DC-00347 and MH44043.

412.9

DIFFERENTIAL REGULATION OF GLUTAMIC ACID DECARBOXYLASE (GAD) AND TYROSINE HYDROXYLASE (TH) mRNA LEVELS IN FUNCTIONALLY-DEAFFERENTED RODENT OLFACTORY BULB. D.M.Stone[1], M.Grillo*[2], F.L.Margolis[2], H.Baker[1] and T.H.Joh[1], [1]Cornell Univ. Med. Coll., White Plains, NY, 10605, [2]Roche Inst. Mol. Biol., Nutley, NJ, 07110.

Expression of the dopaminergic phenotype in olfactory bulb (OB) juxtaglomerular (JG) neurons is dependent upon functional afferent receptor cell innervation. In rodents, destruction of the olfactory epithelium by intranasal irrigation with $ZnSO_4$, or unilateral odor deprivation in neonates, results in a profound decrease in OB TH activity, immunoreactivity and mRNA, when assesed 20-40 days after treatment. At this time, the preservation of immunoreactivity for GABA, a neurotransmitter colocalized with dopamine in JG neurons, suggests that the cells do not die but alter their phenotype. The current study was designed to further characterize the transneuronal effect of functional OB denervation on OB mRNA levels and to determine whether region- or cell type-specific alterations might occur in OB GAD expression. CD-1 mice were intranasally irrigated with 100 μl of 0.17M $ZnSO_4$ or saline (control) and sacrificed 3 weeks later. Neonatal Sprague-Dawley rats were subjected to unilateral naris cauterization and sacrificed 40 days after treatment. OBs were processed for TH and GAD *in situ* hybridization, Northern blot analysis, or immunocytochemistry. Random-primed-labelled fragments of rat TH cDNA or mouse GAD cDNA (provided by R. Greenspan and G. Szabo) were used as probes. By both Northern and *in situ* analyses, JG TH mRNA levels were greatly reduced in both $ZnSO_4$-deafferented (4% of control) and odor-deprived (56% of control) bulb, whereas JG GAD mRNA remained essentially unchanged in both rodent models. Since GAD is found in nearly all dopaminergic OB cells, the preservation of GAD message implies a differential regulation by olfactory receptor cells of TH and GAD gene expression. Supported by grants #DC00336 and MH44043.

412.10

VASOACTIVE INTESTINAL POLYPEPTIDE (VIP) CAUSES INTRACELLULAR CALCIUM OSCILLATIONS IN ASTROCYTES. J. T. Russell, A. Fatatis, P.G. Nelson, and D. E. Brenneman, LDN, NICHD, Bethesda, MD 20892., and Instituto di Farmacologia, II Policlinico, Napoli, Italy.

Previous studies have shown that low concentrations of VIP release neurotropic substances from astrocytes in culture (J. Neurosci. Res. 25: 386, 1990). We have examined the effect of similarly low concentrations of VIP on cytosolic free calcium concentration in astrocytes from new-born rats in culture by monitoring the fluorescence of the calcium indicator, fura-2 using high resolution video microscopy. The cultures were made up primarily of type I cortical astrocytes. Intracellular calcium ion concentration rapidly rises (upto two fold) upon perfusion with VIP (0.01 to 0.1 nM) in most astrocytes. The initial rise is followed by prolonged oscillations in $[Ca^{2+}]_i$ in some cells. In others the rapid rise was followed by steady, elevated intracellular calcium levels. The oscillations were of constant frequency (period = 11 ± 4 sec) and amplitude. Upon removal of agonist the $[Ca^{2+}]_i$ levels ceased to oscillate and returned to resting levels. In calcium free EGTA containing (1 mM) Ringer solutions, although the initial rise was smaller, the oscillations still persisted suggesting that they are caused by release of calcium from intracellular stores. This elevation of intracellular free calcium may participate in the mechanism by which VIP receptor activation causes secretion of factors necessary for neuronal survival from astrocytes.

412.11

INCREASE IN SURFACE ACETYLCHOLINE RECEPTOR IN L5 CLONED MUSCLE CELLS INDUCED BY ASCORBIC ACID IS NOT MEDIATED BY COLLAGEN. E. Liu*[1], R. Minor*[2], J. Wootton*[2], T. Podleski[1], and M. Salpeter[1]. [1]Neurobiology & Behavior, [2]Vet. Pathology, Cornell University, Ithaca, NY 14853.

Ascorbic acid (Asc) is the active component of fetal brain extract that induces increased acetylcholine receptor (AChR) expression in myotubes of the L5 rat clonal muscle cell line (Knaack, Shen, Podleski & Salpeter, J. Cell Biol. 102:795, 1986). The induction of AChR expression, as determined by binding of I-125-α-bungarotoxin, occurs with a delay of 20h (Horovitz, Knaack, Podleski & Salpeter, J. Cell Biol. 108:1823, 1989). Furthermore, we find that the delayed increase in AChR can be triggered by a 5 hour exposure to Asc. These studies suggest that an intermediary compound(s) may be involved.

Asc has also been shown to increase collagen secretion in muscle cell cultures (Kalcheim, Vogel, Duksin, PNAS 79:3077, 1982). We investigated regulation of collagen by Asc and asked whether collagen could be an intermediate in the Asc induced upregulation of surface AChR in the L5 cells. We find that collagen increases in response to Asc (2 fold by 6 hours), well in advance of the increase in surface AChR expression. When pepsin digested media from cultures labeled with ^{3}H-proline were assayed on SDS-PAGE, we identified types I, III and V collagens as the soluble species that increase in response to Asc. We and others have shown that the mononucleated cells and not the myotubes are the source of the collagen in muscle cultures. Therefore if collagen mediated the AChR response, that mediation would need to be via collagen secreted into the culture medium. When ultrapure bacterial collagenase was added to the cultures together with Asc, secreted soluble collages were eliminated from the culture media but surface receptors were still elevated to the same extent as produced by Asc alone. From these experiments, we conclude that Asc increases collagen secretion and surface AChR expression independently. Secreted collagen is therefore not an intermediate in the increased expression of AChR.

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412.12

BUTYRATE-INDUCED DIFFERENTIATION OF PC12 CELLS. K. Spiegel. Parke-Davis Div. of Warner-Lambert Co., Ann Arbor, MI 48105.

Previous work by many laboratories has demonstrated that N^6,2'-O-dibutyryl cyclic AMP (Bt_2cAMP) can produce a differentiation of PC12 cells similar to that caused by treatment with nerve growth factor (NGF): cell division slows, neurites are elongated and choline acetyl transferase (ChAT) activity is increased. The present study examines the role of the butyrate moiety in the actions of Bt_2cAMP on PC12 cells. Treatment of PC12 cells for 7 days with n-butyric acid (320 μM-3.2 mM) produced a modest outgrowth of short, thick neurites and an induction of ChAT activity similar to that produced by NGF. Bt_2cAMP produced a similar growth of short neurites and a comparable increase in ChAT. In addition, cotreatment of cultures with NGF and either butyrate or Bt_2cAMP produced a much greater than additive increase in ChAT as well as potentiated neurite outgrowth. In contrast, treatment of cultures with 8-bromo-cyclic AMP produced only a modest increase in ChAT activity and a greatly reduced potentiation of NGF. These results suggest that the butyrate moiety of Bt_2cAMP contributes to the actions previously attributed to cyclic AMP.

412.13

ASSESSMENT OF NEUROTROPHIC ACTIVITY OF A BROAD SPECTRUM OF PHARMACOLOGIC AGENTS IN PC12 CELLS. C. Bay, M.R. Dickerson, R.E. Davis and K. Spiegel. Parke-Davis Div. of Warner Lambert Co. Ann Arbor, MI 48105.

The PC12 cell line, a rat pheochromocytoma, responds to nerve growth factor (NGF) by differentiating into sympathetic neuron-like cells. Cell division slows, neurites are extended and expression of choline acetyltransferase (ChAT) is increased in response to NGF exposure. This provides an in vitro model system for detection of NGF-mimetic or -modulatory activity. In order to determine the specificity of these responses, the effects of agents with known mechanisms of action were examined. PC12 cells were treated with either test compounds alone to see if they possess NGF-mimetic properties or in combination with NGF to look for modulatory actions. After seven days, a qualitative assessment of neurite outgrowth was made and cultures were then harvested and assayed for ChAT activity. Agents acting at cholinergic, catecholaminergic, glutamate, GABA, adenosine, and opiate receptors were tested. In addition, the effects of a series of neuropeptides, polyamines, phosphodiesterase inhibitors, phorbol esters, cyclic nucleotides, and growth factors were examined. Only fibroblast growth factor, butyrate, and cyclic AMP analogs increased levels of ChAT activity. All three agents produced neurite outgrowth, although the neurites produced with the individual treatments differed qualitatively. The same compounds and cyclohexyladenosine were found to potentiate NGF's effects on both neurite outgrowth and ChAT activity.

412.14

STAUROSPORINE DIFFERENTIALLY INFLUENCES NEURITE OUTGROWTH INDUCED BY NGF OR FGF IN PC12 CELLS. M.R. Dickerson, R.Cowmeadow, C. Bay, R.E. Davis and K. Spiegel. Dept. Pharmacology, Parke-Davis Pharmaceutical Research Division, Warner-Lambert, Ann Arbor, MI 48105.

Cultures of PC12 cells were grown in the presence of nerve growth factor (NGF) or basic fibroblast growth factor (FGF), either alone or in combination with staurosporine, a protein kinase inhibitor. Cultures were evaluated for neurite outgrowth at 24 hrs, 3, 5, and 7 days. Cells receiving either growth factor alone underwent rapid transformation producing neurites in a dose and time dependent manner. Cells grown in the presence of staurosporine alone (3 nM - 30 nM) produced neurites that were qualitatively different from those produced in response to NGF or FGF. Initially, staurosporine induced neurites which were longer and more numerous than those induced by either NGF or FGF; this response appeared to be concentration dependent. However, within 3 days in culture, the staurosporine-induced neurites had begun to retract. This subsequent retraction of the staurosporine-induced neurites was also related to concentration. When added in combination with either growth factor, staurosporine initially potentiated neurite outgrowth; however, over time, staurosporine inhibited neurite outgrowth in NGF treated cultures. In contrast, staurosporine potentiated neurite outgrowth induced by FGF in a dose dependent manner at all time points examined. These results suggest that nerve growth factor and fibroblast growth factor promote the formation of neurites in PC12 cells via different or divergent pathways.

412.15

PARACRINE RELATIONSHIP BETWEEN ASTROGLIA AND MICROGLIA IN CULTURES. C. Hao*, L.J. Guilbert* and S. Fedoroff. Department of Anatomy, University of Saskatchewan, Saskatoon, Sask., Canada S7N 0W0 and Red Cross Blood Transfusion Service, Edmonton, Alta. Canada T6G 2R8.

We believe that macrophage-like cells originating in nutritionally deprived astroglia cultures are counterparts of resident microglia in the CNS (Hao et al., Int. J. Dev. Neurosc. in press). Such microglia contain proto-oncogene c-fms, which codes for colony-stimulating factor-1 (CSF-1) receptor. Under normal culture conditions, microglia do not release CSF-1. However, astroglia in cultures release between 100-300 units/ml of CSF-1 but do not express CSF-1 receptor. In the presence of 1 ng/ml of bacterial cell wall lipopolysaccharide (LPS), microglia produce CSF-1 and tumor necrosis factor (TNFα). Neither LPS nor IFN-γ appreciable upgrade the secretion of CSF-1 in astroglia. We found that TNFα is antagonistic to the CSF-1 effect on microglia. This effect is quite different from that observed on bone marrow macrophages.

We propose that under normal culture conditions microglia depend for survival and maturation on CSF-1 paracrine signalling by astroglia but in the presence of LPS and/or IFN-γ microglia become independent of astroglia by developing CSF-1 autocrine signalling. In such conditions microglia seem to be regulated by positive CSF-1 and negative TNFα signalling.

This work was supported by MRC Canada Grant MT4235 (S.F.) and a grant from the NCI (Canada) (L.J.G.).

412.16

DIFFERENT CONCENTRATION REQUIREMENTS OF ADULT AND DEVELOPING DORSAL LATERAL GENICULATE NUCLEUS (dLGN) NEURONS FOR A NEURON SURVIVAL FACTOR. K.L. Eagleson, F. Haun and T.J. Cunningham. Dept. of Anatomy, Medical College of Pennsylvania, Philadelphia, Pa. 19129.

We have previously identified a neuron survival factor that is able to promote the survival of dLGN neurons following visual cortex lesions. The factor is contained in a macromolecular fraction of medium conditioned by explants of the embryonic primordia of the geniculocortical pathway and is concentration specific for two distinct populations of dLGN neurons (defined by their time of origin). After lesions in infant rats, earlier generated neurons (labeled with ^{3}H-thymidine on E14) require a 200-fold lower concentration of the fraction for maximal survival compared to later generated neurons (labeled on E15/16). The distinct peaks of activity for these two populations of neurons in the neonate is no longer observed in the adult. Both E14 and E15/16 neurons could be rescued over the entire 200-fold range of concentrations tested in the infant. Thus, the neurotrophic factor retrieved from these co-cultures is effective for both populations of dLGN neurons over broader and overlapping concentrations in the adult, compared to restricted and non-overlapping concentrations in the neonate. The results may reflect the strict neurotrophic requirements of young neurons during critical developmental stages.

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412.17

BASIC FIBROBLAST GROWTH FACTOR (bFGF) PROMOTES ENTRORHINAL LAYER II CELL SURVIVAL AFTER PERFORANT PATH AXOTOMY. B. J. Cummings and C. W. Cotman, Department of Psychobiology, University of California, Irvine, CA 92717.

The entorhinal cortex is the primary source of afferents to the hippocampus and is a foci of degeneration in Alzheimer's Disease. In an animal model of entorhinal cell loss, axotomy of medial entorhinal cortical fibers projecting to the dentate gyrus of the hippocampal formation via the perforant path leads to selective retrograde cell loss in layer II of entorhinal cortex. We have previously demonstrated that ventricular infusion of basic fibroblast growth factor (bFGF) spares fimbria-fornix axotomized septal cholinergic neurons from atrophy. In the present study, we examined whether chronic bFGF infusion could spare layer II entorhinal stellate cells. Fourteen days after unilateral transection of the perforant path, a 25% loss of Nissl stained cells was detected in the ipsilateral layer II of medial entorhinal cortex relative to the contralateral side. In addition, many weakly stained, achromatic cells were also observed on the transected side. In contrast, no detectable changes in the number of cells were found in layer IV, a cell layer which does not project to the dentate gyrus. Intraventricular infusion of 5 µg/ml bFGF at 5 µl/hour over 14 days via an Alzet miniosmotic pump ameliorated layer II cell loss to less than 5%. In addition, the number of achromatic, weakly stained cells was also reduced. Thus, ventricular infusion of bFGF can prevent cell loss associated with retrograde degeneration in the entorhinal cortex following axotomy of the perforant path. These findings could lead to a better understanding of the utilization of trophic factors in neurodegenerative disease.

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412.18

RETARDATION OF SYMPATHETIC NEURONAL OUTGROWTH IN CULTURE BY β AMYLOID ISOLATED FROM ALZHEIMER'S DISEASE BRAINS. A.E. Roher*, M.J. Ball, S.V. Bhave, T.D. Wakade* and Arun R. Wakade. Depts. of Anatomy/Cell Biology and Pharmacology, Wayne State University, Detroit, MI, and Dept. of Pathology, University of Western Ontario, London, Canada.

We have examined the effects of β amyloid(βA) isolated from patients with Alzheimer's disease on the survival and neurite outgrowth of peripheral sympathetic neurons(SN) of chick embryo and neonatal rat. The neuritic plaque core proteins (NPCP) were isolated, solubilized in 80% formic acid, precipitated by dialysis against guanidine-HCl and fractionated by Superose 12 size exclusion HPLC. The βA containing fraction was dialyzed against betaine. Dissociated SN were cultured in F14 medium supplemented with insulin and transferrin (1 µg/ml) and NGF (40 ng/ml), or phorbol 12,13-dibutyrate (100 nM). βA (5-60 µg/ml) caused dose-dependent reduction in the survival of both types of SN in 24 to 48 hrs. Higher concentrations (50 µg/ml) killed SN, whereas lower concentrations (20 µg/ml) arrested the neurite outgrowth. After washout of lower concentrations of NPCP and βA, SN resumed their normal growth. When SN were cultured for 2 days and then exposed to 50 µg/ml NPCP, there was gradual loss of neurites followed by disintegration of cell bodies. We conclude that βA of Alzheimer's disease apparently is capable of arresting the growth of sympathetic neurons. (Supported by NIH HL18601, AG03047 and Atkinson Foundation.)

412.19

ACETYLCHOLINE RECEPTOR-INDUCING ACTIVITY DOES NOT INCREASE INTRACELLULAR C-AMP IN CHICK MYOTUBES F.A. Johnson, D.L. Falls, G.D. Fischbach Dept of Anatomy and Neurobiology, Washington University School of Medicine, St Louis, MO, 63110

ARIA, AChR-Inducing-Activity, is a 42kD glycoprotein purified from chicken brain that increases the synthesis of AChRs in chick myotubes, and may play a role in the development of the neuromuscular junction. We are interested in second messengers that might mediate this effect. Drugs that increase intracellular cAMP also increase the synthesis of AChRs. Therefore ARIA may act through increasing cAMP. Fibroblast-free cultures of chick myotubes were treated with ARIA, CGRP, a small peptide that increases the number of AChRs and cAMP levels (Fontaine et al, J. Cell Bio. 105 1337-1342), or Forskolin, an activator of adenylate cyclase. The cells were harvested at various times from 5 mins to 24 hrs after drug treatment and cAMP measured by a radioimmunassay sensitive to 2 fmol. Forskolin, at 100 uM, increased cAMP levels 17-fold, reaching the half maximal effect before 5 min. CGRP increased cAMP 4.5-fold over a similar time course. ARIA produced no detectable increase. In parallel plates used to assay AChR insertion rate, measured by ^{125}I-bungarotoxin binding, ARIA increased receptor incorporation 3.2-fold, Forskolin, 4.3-fold, and CGRP increased it by 10%. Furthermore, in separate experiments, the effects of ARIA and Forskolin, and ARIA and CGRP were additive. As ARIA had as large effect on receptor incorporation as Forskolin and larger than CGRP and caused no detectable increase in cAMP levels, we conclude that ARIA does not act via a cAMP-dependent mechanism.

412.20

THE DOPAMINE CONTENT OF IMMORTALIZED HYBRID NEURONS IS DEPENDENT ON THE PRESENCE OF TARGET TISSUE. H.K. Choi, L.A. Won, and A. Heller Dept. of Pharmacological and Physiological Sciences, The University of Chicago, Chicago, IL 60637

We have previously reported the generation of hybrid cell lines by fusion of cells from the mouse rostral mesencephalic tegmentum at embryonic day 14 (E14) with a neuroblastoma cell line (N18TG2) (Choi et al., *Soc. Neurosci. Abstract*, Vol.14:319, 1988). One of the hybrid cell lines containing a high level of dopamine (DA) was shown to have neuronal properties including immunoreactivity to neurofilament proteins, large voltage sensitive sodium currents, and formation of aggregates in a rotation-mediated cell culture. The DA-containing neurons found in substantia nigra normally innervate telencephalic structures including corpus striatum (CS), frontal cortex (FCx), and occipital cortex (OCx). Tectum (T) or thalamus (TH) do not receive a DA innervation. Two thousand DA-hybrid cells were coaggregated with 8 million dissociated CS, FCx, OCx, T, or TH cells microdissected from E14 embryos in a 3-dimensional cell reaggregation system (Hemmendinger et al., *PNAS*, 78:1264, 1981). The cultures were treated with 1 mM n-butyrate from day 2 to 7 to suppress overgrowth of the hybrid cells. At day 7, the aggregates were processed for measurement of endogenous DA content by HPLC and for visualization of the hybrid cells by catecholamine-induced histofluorescence and for light microscopy by cresyl violet. The concentration of DA (ng/mg protein) in various coaggregates was CS: 21.1 $\pm$ 3.6, FCx: 12.1 $\pm$ 1.6, OCx: 20.2 $\pm$ 2.4, T:1.2 $\pm$ 0.4, and TH: 0.8 $\pm$ 0.2. Cresyl violet stained sections showed approximately equal numbers of hybrid cells in all cases. Brightly fluorescent DA hybrid cells were visualized only in coaggregates containing the target tissue. The results demonstrate that DA-containing hybrid cells derived from the mesencephalic dopaminergic neurons respond selectively to the presence of target tissue. Supported by MH28942 and GM07151.

412.21

ACTIVITY REGULATES EXPRESSION OF MAJOR HISTOCOMPATIBILITY COMPLEX CODED MOLECULES IN RAT SKELETAL MUSCLE. K. Gundersen and J. Mæhlen. Inst. of Neurophysiology and Physiology, Univ. of Oslo, N-0162 Oslo 1, Norway.

Molecules coded by the Major Histocompatibility Complex (MHC) play a prime role in immunological recognition. In normal nerve and muscle tissue these molecules are expressed at very low levels, but are up-regulated during local immunological reactions when lymphocytes invade the tissue and release certain MHC-inducing cytokines. Recently expression of MHC-molecules was described in denervated skeletal muscle (Mæhlen, J., *Brain Res.*, 481:368, 1989). We have observed that this phenomenon also occur in athymic animals suggesting that lymphocytes are not involved. Now we ask whether the nerve-evoked action potential activity in skeletal muscle may serve as a regulator of MHC-expression.

MHC molecules were immunostained on cryosections from soleus muscles after 5-14 days of either i) tetrodotoxin impulse blockade of the sciatic nerve or ii) direct electrical stimulation of denervated muscles. Inactivity was similar to denervation in causing MHC class I expression along the muscle fibre surface and in the sarcoplasm, and in causing MHC class II expression on cells located in the interstitium of the muscle. Moreover, the MHC up-regulation in denervated muscles was strongly reduced by electrical stimulation.

In conclusion, our data show that MHC class I expression is suppressed by evoked action potential activity in normal muscle cells, and suggest that inactive muscle cells produces a signal that leads to apparence of MHC class II positive cells in the interstitium .

OTHER TROPHIC AGENTS II

413.1

INSULIN-LIKE GROWTH FACTOR I ACTIVATES A PHOSPHOTYROSINE-CONTAINING MICROTUBULE ASSOCIATED PROTEIN-2 KINASE IN BOVINE CHROMAFFIN CELLS. A.L. Cahill and R.L. Perlman. Depts. of Pediatrics and Pharmacol. and Physiol. Sciences, The University of Chicago, Chicago, IL 60637

Previously this laboratory reported that bovine chromaffin cells have insulin-like growth factor I (IGF-I) receptors and that these receptors are protein tyrosine kinases (Dahmer et al., J. Neurochem. 53, 1036, 1989). To characterize the endogenous substrates for this kinase we have isolated phosphotyrosine-containing proteins from bovine chromaffin cells and have identified one of them as an IGF-I activated microtubule associated protein-2 (MAP-2) kinase. Phosphotyrosine-containing proteins were isolated from control and IGF-I treated cells by adsorption to and specific elution from antiphosphotyrosine-Sepharose beads. When eluates were assayed for MAP-2 kinase activity, increased activity was found in eluates from IGF-I treated cells. The activation of MAP-2 kinase by IGF-I varied with the time of treatment (maximal at 10-15 min) and concentration of IGF-I (maximal at 10 nM) and was potentiated by the tyrosine phosphatase inhibitor Na_3VO_4. Myelin basic protein was also a good substrate for this kinase, but histones H-IIS and H-IIIS and ribosomal S6 protein were not phosphorylated. Treatment of the MAP-2 kinase with alkaline phosphatase abolished the kinase activity indicating that phosphorylation of the MAP-2 kinase is required for its activity. The IGF-I stimulated MAP-2 kinase described here is similar to the insulin-stimulated MAP-2 kinase described by Ray and Sturgill (J. Biol. Chem. 263, 12721, 1988) and may participate in an IGF-I activated kinase cascade similar to that proposed for transduction of signals from the insulin receptor. (Supported by NIH grants HL29025 and HD04583 and NSF grant BNS-8820342)

413.2

EFFECT OF CALCITONIN GENE-RELATED PEPTIDE ON NEURITE GROWTH OF CENTRAL NEURONS IN CULTURE. Fu-Zhuang Wang, Aishi Ding*, Jia Chen* and Xiaping Xie*, Dept.of Neurobiology, Institute of Basic Medical Sciences, Beijing 100850, China

Effects of calcitonin gene-related peptide on neurite growth were studied in primary dissociated spinal cord cell cultures from 12-14 day mouse embryos and hippocampal neurons from new born rat. Traditional cell cultures were made at density of 5×10^5 cell/ml in the culture medium containing 95% MEM and 5% horse serum added nutrient supplement. β-hCGRP(10ng/ml) were added to culture dishes conaining 2ml cell suspensions. The neurite outgrowth was assessed at 24 hr. Both spinal cord cells and hippocampal neurons were grown in the presence of CGRP, the numbers and length of neurite outgrowth were markedly incresed twice as much as that seen in the control cultures ($p<0.01$). From long-term cultures, the cell size and the number of surviving neurons were, on the average, higher than those observed in the parallel series grown with non-CGRP medium.

To quantitate the effects of the neuronal growth the total content of cellular protein were analyzed by flow cytometry. For cytometric analysis of cellular protein, control cells and CGRP-treated neurons by 12 days and 15 days were stained with fluorescein isothiocyanate (FITC) and fluorescence was measured on a single cell basis. From cellular fluorescence histograms, it was shown that the relative content of cellular protein was elevated in presence of CGRP. These results show that CGRP dramatically enhance the neural outgrowth and is involved in protein synthesis in cells.

413.3

EFFECT OF HEPARIN BINDING GROWTH FACTORS ON THE LA-N-1 NEUROBLASTOMA CELL LINE S.Sullivan1, T. Maciag2, R. Forough2, X. Zhan2, and M.F.D. Notter1. 1Univ. of Rochester Sch. of Med., Dept. of Neurobiology and Anatomy, Rochester, NY 2Laboratory of Molecular Biology, American Red Cross, Rockville, MD

The heparin binding growth factors (HBGF) are a class of related polypeptides which include acidic fibroblast growth factor (aFGF also HBGF-1) and basic FGF (bFGF also HBGF-2). These factors have varied effects in different tissues, including neural tissue. We evaluated the effects of various concentrations of aFGF and bFGF in vitro using the adreneric/serotonergic human neuroblastoma cell line LA-N-1. Neither affected the rate of cellular division; however, both factors stimulated the cells to differentiate morphologically, as seen by the extention of neurites.

We also tested altered forms of HBGF: HBGF-1a, aFGF with residues 1-20 truncated from the NH2 terminus domain; HBGF-1U which lacks residues 21-27, a putative nuclear translocation sequence; and HBGF-1U2, which also lacks 21-27, but has a yeast histone translocation sequence at the NH2 terminus. HBGF-1a and HBGF-1U2 were similar to unaltered HBGF-1 while HBGF-1U had no effect on cellular morphology. Mixing equal concentrations of HBGF-1U and HBGF-1U2 did not inhibit the neurite producing ability of HBGF-1U2.

It has been suggested that aFGF increases the amount of intermediate filament in a cell while bFGF increases the microtubule content. To examine the possibility that this occurs in LA-N-1 cells, cultures were stained for neurofilament protein and tubulin. We were unable to distinguish a clear difference in staining pattern between aFGF or bFGF treated cultures. We are currently examining these cultures by electron microscopy to gain a more definitive answer since microtubles and intermediate filaments are distinguishable at the EM level.

Supported by NIH NS25778 and Predoctoral fellowship NIMH 09851

413.4

STIMULATION OF PROLIFERATION OF C6 GLIOMA CELLS BY S100ß: EVIDENCE FOR A CENTRAL NERVOUS SYSTEM GROWTH FACTOR. S.W. Barger*, R.H. Selinfreund, and L.J. Van Eldik. Depts. of Cell Biology and Pharmacology, Vanderbilt University, Nashville TN 37232.

S100ß is a member of a family of homologous calcium binding proteins, and promotes the survival and morphological differentiation of embryonic cortical neurons. S100ß production in brain is predominantly in glial cells, including the rat C6 glioma cell line which synthesizes and secretes the protein. Specific reductions in S100ß in C6 cells by antisense oligonucleotides resulted in a reduced growth rate. Addition of a recombinant S100ß to arrested, subconfluent cultures stimulated proliferation of C6 cells in a dose-dependent manner. Exogenous S100ß also stimulated [^{3}H]-thymidine labeling of nuclei and the expression of protooncogenes. This stimulation of proliferation by S100ß complemented the effects of PDGF but not insulin. S100ß was unable to stimulate proliferation of two neuroblastoma cell lines. These data suggest the possibility that S100ß acts in an autocrine manner to coordinate the growth of glial cells with the differentiation of neurons in the developing brain. (Supported by American Paralysis Association and Cystic Fibrosis Foundation).

413.5

PURIFICATION AND CHACTERIZATION OF RECOMBINANT BOVINE BASIC FIBROBLAST GROWTH FACTOR FROM ESCHERICHIA COLI Z.Y. Yu and G. Wang*, Department of Molecular Neurobiology, China Rehabilitation Res, Ctr. Box 2619, Beijing 100077, China

The cDNA sequence coding for bovine basic fibroblast growth factor (bFGF) has been cloned into an expression vector (pRC 29) downstream of the *Lac* Z promoter. The DNA sequencing results have been shown that the bFGF cDNA is inserted in the correct reading frame of the promoter. The expression of the fusion gene in *Escherichia Coli* can lead to an accumulation of about 15 mg recombinant bFGF per liter bacteria culture. The recombinant bFGF with purity of about 95% has been obtained by passing the bacterial lysate through an ion exchange column and a heparin affinity chromatography. The bFGF so obtained has been shown to have biological activities indistinguishable from the natural bFGF purified by us from bovine pituitary, either in mitogenicity or in angiogenesis assays. Animal experiments have been shown that the bFGF can promote the soft tissue wound healing and may have antiphlogistic effect. Injection of the recombinant bFGF into the shaved rabbit ear not only induced new blood vessel growth, but also promoting hair growth in the region of injection, indicating that the bFGF might be of value in the stimulation of regrowth of hair.

413.6

CLONING AND EXPRESSION OF A cDNA ENCODING A NOVEL HUMAN NEUROTROPHIC FACTOR. Y. Kaisho*, K. Yoshimura*, K. Nakahama*, and #K. Kato. Biotechnol. Res. Labs., and #Biol. Res. Labs., Takeda Chem. Ind., Ltd., 17-85, Jusohonmachi 2-chome, Yodogawa-ku, Osaka 532, Japan.

Two structurally related neurotrophic factors, nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF), were reported which are believed to play an essential role in the growth, survival, and differentiation of neurons in the nervous system.

To obtain other NGF related genes, we tried to screen a human glioma cDNA library under low stringent hybridization conditions using a synthetic DNA corresponding to human NGF as a probe. A cDNA has been cloned which encodes a novel human neurotrophic factor (designated nerve growth factor-2, NGF-2) composed of 257 amino acid residues including a prepro-sequence of 138 residues and a mature region of 119 residues. The amino acid sequence of NGF-2 exhibited 58% and 55% similarity with human NGF and pig BDNF, respectively. The conditioned medium of COS-7 cells transfected with an expression plasmid for the NGF-2 cDNA supported the survival of sensory neurons isolated from dorsal root ganglia of embryonic chicks. Upon Northern blotting analysis, large amounts of NGF-2 mRNA were detected in all the tissues examined except muscle and testis, the highest level of the mRNA synthesis being observed in kidney. In rat brain, NGF-2 mRNA was detected at embryonic day 17.5, and the level of the mRNA synthesis reached a maximum 1 to 2 weeks postnatally.

These results suggest that there are at least three nerve growth factor related genes, NGF, NGF-2, and BDNF. These factors may have distinct functions in vivo, because they showed different tissue distribution and developmental expression.

413.7

INSULIN-LIKE GROWTH FACTOR II GENE EXPRESSION IN HUMAN RETINAL PIGMENT EPITHELIAL CELLS. D.M. Martin, P.J. Venta* and E.L. Feldman. Departments of Neurology and Human Genetics*, University of Michigan Medical Center, Ann Arbor, MI 48109.

Diabetic retinopathy is the major cause of blindness in the United States. Recent observations implicate metabolically induced neovascularization in diabetic retinopathy. Insulin-like growth factors, known to stimulate endothelial cell proliferation, have been found in the vitreous and may act directly within the eye. We postulated that insulin-like growth factor II (IGF-II) is produced within the eye and may play a role in the pathogenesis of diabetic retinopathy. We report that cultured human retinal pigment epithelial (HRPE) cells express the IGF-II gene. We are currently investigating changes in IGF-II gene expression in HRPE cells conditioned in high glucose media.

A radiolabeled antisense RNA transcript was generated from the T7 promotor of the pGem-4 vector (Promega) containing 854 bp of a human IGF-II cDNA. Total cellular RNA was isolated by the guanidinium thiocyanate-phenol method and separated by formaldehyde-agarose gel electrophoresis. RNA was subjected to Northern blot analysis on nylon membranes and hybridized with the radiolabeled RNA probe. No cross-hybridization of the IGF-II probe to human IGF-I template DNA was seen. Autoradiography revealed a 1.2 Kb transcript which co-migrated with a 1.2 Kb transcript present in fetal rat muscle. To our knowledge, this is the first report of IGF-II gene expression in HRPE cells. The presence of IGF-II in HRPE cells suggests that this growth factor may play a role in the pathogenesis of diabetic retinopathy.

(Supported by grant NS01381 to ELF)

413.8

COMPARATIVE STUDIES OF THE EFFECTS OF SECOND MESSENGER SYSTEMS ON THE DEVELOPMENT OF LOCUS COERULEUS NEURONS. L.Sklair and M. Segal. Center for Neuroscience, The Weizmann Institute of Science, Rehovot 76100, Israel.

Locus coeruleus (LC) cells are remarkably plastic in vivo. In LC cultures noradrenergic (NA) cells differ strikingly from the other neurons in their extensive neuritic arborization. Intracellular messengers play a role in regulating neurite outgrowth and cell survival. In the present study we compared the effect of adenylate cyclase (AC) activation and Ca^{2+} entry blockade on the development of NA cells versus glutamatergic (GLU) cells in LC cultures. Maturation of NA and GLU cells was assessed by measuring high affinity uptake of [^{3}H]-norepinephrine (NE) and [^{3}H]-D-aspartate (D-asp) respectively. Treatment of cultures with the AC activator forskolin (1uM) or directly with d-Bu-cAMP (1mM) for 4-5 days increased [^{3}H]-NE uptake by 90 and 30%, respectively. Blocking Ca^{2+} entry into the cell with La^{3+} (1uM) increased [^{3}H]-NE uptake by 83%. All these treatments did not affect [^{3}H]-D-asp uptake. Thus, the greater sensitivity of noradrenergic cells to these developmental cues may explain their unique plastic properties.

413.9

CHARACTERIZATION AND PARTIAL PURIFICATION OF A NOVEL NEUROTROPHIC FACTOR SECRETED FROM HUMAN FETAL TESTIS CELLS. M.S. Huynh, R.H. Soriano & D.G. Roufa. CNS Disease Research, Searle R&D c/o Monsanto, St. Louis, MO 63198

Neuronal survival factors modeled after the prototypic factor NGF, are molecules secreted by the target and affect the neurons innervating that target. We report here, the characterization and partial purification of neurotrophic activity from N2 medium conditioned by human fetal testis cells.

The testis factor was active in neuronal survival assays of dissociated SCG and nodose ganglion and inactive in DRG bioassay. It was inactive in PC12 cells neurite outgrowth assay. Neither rabbit anti-mouse NGF nor anti-human NGF sera blocked its SCG survival activity. The addition of mouse NGF to the testis factor gave additive effect.

The activity was purified 1500-fold with CM-cellulose at pH 6.8 following concentration and dialysis. Its native molecular mass was 35~50 kDa, showing a broad active peak on Sephacryl S-200. This factor is likely to be glycosylated as the activity adsorbs to Con A and WGA.

Thus, we identified a novel neurotrophic substance with biological and chemical properties distinct from NGF.

413.10

ASTROGLIAL MITOGENS AFFECT SEPTAL CHOLINERGIC CELL EXPRESSION DIFFERENTIALLY.
R.L. Kenigsberg and I.E. Mazzoni* Centre de Recherche Pédiatrique. Hôpital Ste-Justine, Montreal, Que. Canada H3T 1C5.

It has been proposed that the regenerative capacity of CNS neurons may be restricted by proliferating astroglia at a lesion site which poses physical and/or chemical barriers. We previously found that EGF, a potent glial mitogen, when applied to septal cultures from fetal rat brains, produced a decrease in 2 cholinergic cell parameters, choline acetyltransferase. (ChAT) and acetylcholine (ACh) content (Kenigsberg et al., J. Neurochem. 52, Suppl., S192D, 1989). As these changes were accompanied by a marked glial cell proliferation and no change in neuronal survival we extended these studies to determine the specificity of the EGF response. Consequently, we examined the effects of a number of glial mitogens which are released following injury in the CNS on septal cultures. Although all mitogens examined produced astroglia proliferation, only EGF decreased ChAT activity while thrombin produced a significant increase in ChAT and PDGF had no effect. These results suggest that although several mitogens may affect glial cell division similarly, they may control the expression of astroglia and neurons differentially.

413.11

GANGLIOSIDES PREVENT THE INHIBITION BY K252a OF NGF RESPONSES IN PC12 CELLS. G. Ferrari, M. Fabris*, M.G. Fiori, N. Gabellini* and C. Volonté (1). Fidia Research Labs., Abano Terme (PD), Italy and (1) College of Physicians and Surgeons of Columbia University, New York 10032.

K252a, a general kinase inhibitor, selectively blocks the actions of NGF in PC12 cells. Since gangliosides have been reported to modulate neuronal cell responsiveness to NGF and to regulate several protein kinases, the ability of these compounds to revert the inhibition by K252a was assessed. Parameters at both short and long-term times following treatment of PC12 cells with NGF were analyzed, and known to be either transcription-dependent or-independent events. Gangliosides completely prevented the inhibition by K252a of NGF-induced neurite regeneration, c-fos induction, and, partially, protein kinase N activation. The ganglioside protective effects were concentration-dependent and required the intact molecule. These findings raise the possibility that gangliosides might affect a specific pathway of NGF responses sensitive to inhibition by K252a.

413.12

NEURITOGENIC AND METABOLIC ACTIONS OF INDIVIDUAL GANGLIOSIDES AND THEIR POTENTIATION OF TAURINE'S EFFECT ON NEURO-2a CELLS. C.L. Lu, G. Yorke and F.J. Roisen. Department of Anatomical Sciences and Neurobiology, University of Louisville, School of Medicine, Louisville, Kentucky 40292.

Gangliosides play an important regulatory role in the development of the nervous system and may modulate the actions of neurotrophic signals. However, reports of species-specific ganglioside-mediated effects are rare. To examine the neurotrophic responses elicited by individual gangliosides, we examined the neuritogenic and metabolic actions of four major ganglioside species (GM1, GD1a, GD1b, GT1b) in the presence and absence of taurine on the murine Neuro-2a neuroblastoma cell line. Taurine's neurotrophic actions on Neuro-2a cells have been reported recently (Spoerri, Caple, and Roisen, Int. J. Devl. Neurosci., in press). In this study, computer-assisted morphometry was employed to evaluate neuritic sprouting and the number of process bearing cells after 24 hr exposure to the individual gangliosides alone or in the presence of taurine. Furthermore, the activity of ornithine decarboxylase (ODC), the rate limiting enzyme in polyamine biosynthesis, was examined. Exposure of Neuro-2a cells to media containing the individual gangliosides (150 ug/ml) or taurine (1 mM) enhanced neuritogenesis. ODC activity was enhanced 1.6-1.9 fold after 5 hr exposure to GD1a, GD1b, or GT1b. GM1, although neuritogenic, did not enhance ODC activity. Simultaneous treatment of Neuro-2a cells with taurine and each ganglioside potentiated the neuritogenic action of taurine. Furthermore, when submaximal levels of taurine (0.1 mM) were used, GD1a, GD1b, and GT1b elevated ODC levels significantly higher (3.6-4.6 fold) than taurine or the level obtained by the individual ganglioside alone. These studies demonstrate that neuritogenesis and polyamine synthesis are independent and that gangliosides may play a species-specific regulatory role in neuronal development. Supported by NIH NS24524.

413.13

REGULATION OF THE SURFACE DISTRIBUTION OF GM1 BY UNDERLYING CYTOSKELETAL ORGANELLES. I. H. Fentie and F. J. Roisen. Department of Anatomical Sciences and Neurobiology, University of Louisville, School of Medicine, Louisville, Kentucky 40292.

Gangliosides are relatively abundant components of neuronal membranes. Their abnormally high accumulation in neurons due to gangliosidosis results in aberrant neurite formation. Neurons, including the murine Neuro-2a neuroblastoma line, undergo increased neuritogenesis after exposure to the ganglioside GM1. Responses to Nerve Growth Factor-dependent and NGF-independent trophic agents can be potentiated by GM1. Thus evidence is accumulating that gangliosides, especially GM1, play a role in neuronal development, although the mechanism remains unknown. In this study, we examined the surface distribution of GM1 on Neuro-2a cells at different stages of neuritogenic development. GM1 was localized using direct fluorescence of cholera toxin B-FITC and with indirect immunofluorescence and scanning transmission electron microscopy employing antibodies to GM1. Although a uniform distribution of label was found on perikaryal and neuritic surfaces, occasional linear arrangements suggested the possibility of an underlying fibrillar organelle. Cytoskeletal probes were used to determine its nature. Taxol (1.0 uM) treatment of Neuro-2a cells produced neurites with distal GM1 positive label and stabilized microtubules (MTs). In contrast, Colcemid (0.05 ug/ml) produced neurites devoid of GM1 label and lacking MTs. Cytochalasin D (2 ug/ml) reduced microfilaments but had no visible effect on GM1 surface distribution. Semiquantitative studies, in progress, employ the direct fluorescence of cholera toxin B-FITC and cholera toxin-gold conjugates in combination with cytoskeletal altering agents. These studies demonstrate that the surface distribution of GM1 is dependent on microtubule organization. Supported by NIH grant NS24524.

413.14

GM1 GANGLIOSIDE TREATMENT DOES NOT INDUCE AXONAL SPROUTING IN ADULT HAMSTER VISUAL SYSTEM.
B.A. Sabel[1], L. Cavicchioli[2]* and A. Leon[2]. 1. Institute of Medical Psychology, University of Munich Medical School, 8000 Munich 2, Fed. Rep. Germany and (2) FIDIA Research Laboratories, 35031 Abano Terme, Italy.

When hamsters are given lesions of the right superior colliculus (rSC) on postnatal day 1 (P1), the retinofugal fibers from their left eye cross the midline to innervate the wrong, left SC. Right eye removal (rEE) at a time up to P14 will result in sprouting of these fibers in the left SC and treatment with exogenous gangliosides (GM1) enhances this sprouting after rEE at P9 (Sabel, B.A. and Schneider, G., Exp. Brain Res. 71: 365, 1988). We now report the effects of GM1 in a preparation where sprouting does not occur normally (i.e. rEE after P14) or when the sprouting is vigorous (rEE on P1).

Hamsters received rSC lesions on P1, with rEE on P1, P18 or P30. Groups of hamsters (n=7-9 each) were then treated i.p. with 30 mg/kg GM1 (Fidia Res. Labs.) or saline, starting on the day of rEE. After a 4-week period of daily treatment, the retinofugal pathway and left SC innervation was demonstrated by removal of the left eye and staining degenerating axon terminals using the Fink-Heimer method. Analysis of the size of the terminal field in the left SC clearly revealed the previously known decline in sprouting vigor with increasing age, but GM1 was without detectable effect on the extent of sprouting in hamsters with rEE at P1, P18 or P30.

Thus, GM1 does not enhance axonal sprouting when maximal sprouting vigor is present. Also, GM1-treatment does not elicit axon sprouting in the adult hamster visual system, suggesting that GM1 is only effective during an ongoing sprouting response (as at P9).

Supported by DFG grant SFB220/D10 and Fidia Research Labs.

413.15

THE GANGLIOSIDE GM1 PROTECTS AGAINST THE 1-METHYL-4-PHENYL-1,2,3,6-TETRAHYDROPYRIDINE (MPTP) - INDUCED DEGENERATION OF NIGRAL DOPAMINE CELLS IN THE MALE C57BL/6 MOUSE. [1]A.M. Janson, [1]K. Fuxe, [2]L.F. Agnati*, [3]G. Toffano* and [4]M. Goldstein. [1]Dept for Histology and Neurobiology, Karolinska Institutet, S-104 01 Stockholm, Sweden. [2]Dept of Human Physiology, University of Modena, Modena, Italy, [3]Fidia Research Laboratory, Abano Terme, Italy, [4]Dept of Psychiatry, New York University Medical Center, New York, USA.

Previous experiments have shown protective effects of two week's chronic ganglioside GM1 treatment against the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-(MPTP) induced disappearance of tyrosine hydroxylase (TH) immunoreactive (IR) nerve cell bodies and dendrites in the substantia nigra and terminals in the neostriatum of the nigrostriatal dopamine (DA) system in the male C57 Bl/6 mouse (Acta Physiol. Scand. 1988, 132, 587-588).

In this study five different doses of the ganglioside GM1 (3 mg·kg^{-1} to 100 mg·kg^{-1}, i.p.) were given twice daily for two weeks starting 15 minutes after the MPTP injection (40 mg·kg^{-1}, s.c.). Morphometric and densitometric evaluation of TH IR nerve cell bodies and dendrites were performed following the avidin-biotin immunostaining procedure. Applying the Jonckheere-Terpstra test dose-related increases in both the number ($p<0.01$) and the mean area ($p<0.05$) of the TH IR nerve cell body profiles were found compared with the MPTP alone group, whereas the densitometric parameter mean grey value showed a dose-related decrease ($p<0.01$). Similar result were obtained in the analysis of nigral TH IR dendritic profiles.

Thus, chronic treatment with the ganglioside GM1 can markedly counteract the MPTP-induced disappearance of TH IR nigral nerve cell bodies and dendrites. The mechanism underlying this protection, of high clinical interest in the treatment of Parkinson's disease, appear to be multifold, involving e.g. membrane actions and modulation of protein phosphorylation.

413.16

GANGLIOSIDE TREATMENT AND RECOVERY OF FUNCTION AFTER GRADED CRUSH OF THE RAT OPTIC NERVE .

J. Sautter* and B. A. Sabel, Inst. of Medical Psychology, University of Munich Medical School, 8000 Munich 2, Fed. Rep. of Germany (SPON: European Neuroscience Association)

To study recovery of function in a definable model of brain injury, we have previously employed the graded crush of the rat optic nerve as a paradigm (Duvdevani et al., in prep.) Using this paradigm it is possible to simulate variable degrees of axonal injury from "mild" to "severe", ressembling "diffuse axonal injury (DAI)", a primary response of the brain to traumatic injury in humans. We now report the effects of GM1-ganglioside treatment on functional outcome after graded crush of the rat optic nerve using two behavioral paradigms.

In the orienting paradigm, rats have to orient towards a visual stimulus in the visual field ipsilateral to the the unilateral crush. Here, initial loss of function was followed by recovery within about 2 weeks. However, when rats were treated with 50 mg/kg GM1 (i.p.), they performed, on average, better than control rats on all postoperative days, but significant differences were only found on days 11 and 12.

In the brightness-discrimination paradigm, bilaterally crushed rats had to choose between a bright or dark stimulus in a maze to obtain water reward. Although the deficit and subsequent recovery of function is similar to that of the orienting paradigm, GM1 was without detectable effect on brightness-discrimination performance.

These behavioral results can be taken to suggest that ganglioside treatment can reduce visual deficits after graded crush of the rat optic nerve, but a suitable and sensitive behavioral paradigm is needed to demonstrate efficacy of treatment.

413.17

EPIDERMAL GROWTH FACTOR (EGF) ENHANCES, IN RATS, DOPAMINERGIC PATHWAY "IN VIVO" AN IMMUNOHISTOCHEMICAL STUDY A Zecchinelli[1,2], G. Pezzoli[1*], S. Ricciardi[3*], R.E. Burke[2], S. Fahn[2], R. Fusi[3*], C.B. Mariani[1*], G.Scarlato[1*], and A. Carenzi[3*] [1]Inst of Neurol. Univ. Milan, Italy. [2] Neurol. Inst. Columbia Univ. N.Y. N.Y. 10032. [3] Zambon-Group Bresso-Milan, Italy.

Male Sprague Dawley rats received unilateral transection in the left nigro-striatal pathway. 45 days after surgery, animals were infused in the left lateral ventricle by an osmotic Alzet minipump at rate .5 µl/hr. Group I was treated with EGF (mouse), highly purified, (Zambon-Group), group II with heat denatured EGF, group III with vehicle. The infusions lasted 28 days; apomorphine and amphetamine induced rotational behaviort was tested ≈ 2 months after the lesion and at the end of the 3rd and 4th month after the infusion. Animals were then perfused intracardiacally with 4% paraformaldehyde, decapitated and the brains frozen; representative sections (Paxinos Watson) for both the striatum and the substantia nigra were cut on a cryostat and immunoperoxidase stained for tyrosine hydroxylase (TH). Alternate sections were stained for Glial Fibrillary Acidic Protein. Effects on striatal TH staining were assessed blind to experimental condition by two observers by rating the extent of TH positive staining at individual fiber level, for the striatum, on a 5 point scale. The cross-sectional area of TH staining was further determined at population level as percent of the whole striatal area by an Amersham RAS-3OOO image analysis system; staining on the lesioned side was expressed in percent compared to the control side. The number of TH positive neurons in substantia nigra was assessed as a midpoint range of values given by two observers by examination at 40 x. The actual count of neurons was then performed. EGF treatment resulted in a significant increase of the percent of TH-positive staining in the lesioned side compared to both controls. Similar results were obtained in a second group of animals. EGF treatment also resulted in a significant increase of the percent of preserved TH-positive neurons in the substantia nigra, with a high correlation to the degree of preservation of striatal TH-positive staining. We conclude that EGF infusion in the ventricle can partially restore or preserve, perhaps by a trophic effect, the integrity of the dopaminergic nigrostriatal pathway, in rats, after mechanical hemitransection

413.18

EPIDERMAL GROWTH FACTOR IS A MITOGEN AND INCREASES DOPAMINE UPTAKE IN RAT EMBRYO MESENCEPHALIC PRIMARY CULTURE. D. Casper, C. Mytilineou and M. Blum, Fishberg Research Center in Neurobiology and Department of Neurology, Mount Sinai Medical Center, New York, NY 10029.

Epidermal growth factor (EGF) has previously been described as a mitogen on epithelial- and mesenchyme-derived tissue. In the central nervous system, EGF has also been shown to be a mitogen for astrocytes. Our group has previously reported that EGF can increase neuronal and glial dopamine uptake in this culture system (Casper et al., Soc. Neurosci. Abst., 15:708, 1989). In this study, we have placed dissociated mesencephalon from E16 rat embryos into culture. Using chemically defined medium, we have observed that addition of EGF to the cutures can expand a population of cells that can incroporate tritiated thymidine into their nuclei, but do not express the astrocyte markers glial frillary acidic protein (GFAP), the oligodendrocyte marker glactocerebroside (GC), the neuronal mictotubule associated protein tau, or neuron-specific enolase in the presence or absence of the labelled nucleotide. These cells are seen by their large and flattened nuclei, which lightly stain with tau immunocytochemistry, or by their nuclei and scant cytoplasm, seen by phase microscopy. A few cells stain very weakly with antibodies to either GFAP or tau. While this population of "undifferentiated" cells increases, EGF also increases dopamine uptake. Using immunocytochemistry for tyrosine hydroxylase (TH), the rate limiting enzyme in catecholamine biosynthesis to mark dopaminergic cells, we found that the number of cells expressing this marker decreases up to a week in culture, but the number of these cells is stable after this in EGF treated cultures up to 22 days. The number of TH-positive cells in control cultures continues to decrease. Studies to be presented will determine whether the increase in dopamine uptake is due to the action of EGF on the existing stabilized dopaminergic neurons such as inducing neurite outgrowth, or whether it is causing differentiation or neurogenesis of new dopaminergic neurons.

413.19

EPIDERMAL GROWTH FACTOR AND TRANSFORMING GROWTH FACTOR-ALPHA mRNA EXPRESSION IN PCD AND WEAVER MUTANT MICE. L. M. Lazar, K. A. Kelley and M. Blum, Fishberg Research Center for Neurobiology, Mount Sinai School of Medicine, New York, NY 10029.

Our previous investigations into the role of epidermal growth factor (EGF) in the mammalian CNS have focused on the expression of EGF-specific mRNA in normal adult mouse brain. We now report our preliminary findings regarding the changes in level of mRNA expression for EGF and its structural homolog transforming growth factor-alpha (TGF-alpha) in cerebella and lower brainstem of Purkinje cell degeneration (pcd) and weaver mutant mice. Using an RNase protection assay, we have detected elevated levels of EGF mRNA in cerebella of severely ataxic pcd mutant mice (genotypically *pcd/pcd*) as compared to non-ataxic sex-matched littermates. TGF-alpha mRNA levels, however, detected at levels ~80 times higher than that of EGF in the wild-type (*+/+*) cerebellum, are not significantly altered in this mutant model. In contrast, in the cerebella of weaver mutant mice in which the neurodegenerative process is specific to the granule cell population and in which the heterozygous state (*+/wv*) displays cerebellar histopathology intermediate between mutant (*wv/wv*) and homozygous normal (*+/+*) animals, levels of TGF-alpha mRNA are approximately 2.5 and 1.25 times greater for homozygous mutant (*wv/wv*) and heterozygous normal (*+/wv*) cerebella than levels detected in cerebella of homozygous normal (*+/+*) sex-matched littermates. Interestingly, levels of TGF-alpha mRNA in lower brainstem (Medulla-Pons) of the weaver model appear to decrease to 30% and 60% of homozygous normal levels for the homozygous mutant and heterozygous states, respectively. No significant changes in EGF mRNA levels have been observed in cerebella or lower brainstem for the weaver mutant model. Our findings of differential gene expression for EGF and TGF-alpha in these neurodegenerative models, then, suggest that while both neuropeptides utilize a common receptor, their physiological roles in brain may be distinct.

413.20

THE EFFECT OF EPIDERMAL GROWTH FACTOR ON THE DEVELOPMENT OF DOPAMINE NEURONS FOLLOWING EXPOSURE TO THE NEUROTOXIN 1-METHYL-4-PHENYLPYRIDINE (MPP$^+$). T. Bak and C. Mytilineou, Dept. Neurology, Mt. Sinai Sch. Med., New York, N.Y., 10029.

Exposure of mesencephalic cultures to epidermal growth factor (EGF) results in long term stimulation of dopamine (DA) uptake (Casper et al., Soc. Neurosci. Abst., 15:708, 1989). In the present study we examined whether EGF treatment could a) modify the damage caused by the specific neurotoxin MPP$^+$, or b) affect the ability of DA neurons to recover following MPP$^+$-induced damage. Tyrosine hydroxylase (TH) immunostaining and [^{3}H]DA uptake were used to determine DA neuron survival and axon outgrowth. Mesencephalic cultures established from E-16 rats were plated at 5×10^5 cells/dish. EGF (10 ng/ml) in defined medium was added to the appropriate groups and was present throughout the experiment. Cultures were exposed to 10 uM MPP$^+$ for 24 hrs on the 4th day in vitro (DIV). At this time, there was no significant difference between control and EGF-treated cultures with respect to DA uptake and number of TH+ process-bearing neurons. Following removal of MPP$^+$ on 5 DIV, a similar reduction of DA uptake (~80% of controls) and loss of TH+ cells (~45% of controls) was observed in control and EGF-treated cultures, suggesting a lack of a protective effect of EGF against MPP$^+$-induced DA neuron degeneration. The greater effect of MPP$^+$ on DA uptake probably reflects the loss of DA neurons, combined with reduction in neurite length in the surviving TH+ neurons. DA uptake remained lower in the MPP$^+$-treated cultures compared to their respective controls during further in vitro development, but EGF treatment resulted in a pronounced increase in uptake both in control and in MPP$^+$-treated cultures. On 19 DIV the MPP$^+$-treated cultures exposed to EGF had uptake values ~10-fold greater than non-EGF controls. In the control cultures, the number of TH+ process-bearing cells declined on 12 and 19 DIV, but this decline was not observed in EGF-treated cultures. It still remains to be determined whether all of the TH+ cells on 19 DIV are the surviving neurons which were spared from the toxic effect of MPP$^+$. Supported by NIH grant NS-07245, NS-23017 and the United Parkinson Foundation.

413.21

STIMULATION OF ASTROCYTE PROLIFERATION BY PURINE NUCLEOSIDES AND NUCLEOTIDES THROUGH ADENOSINE A2 AND PURINE P2X RECEPTORS. M. Rathbone, P. Middlemiss*, J-K. Kim* and E. Hooftman*. Dept. Biomedical Sci. McMaster University Health Sciences Centre, 1200 Main St. W., Hamilton, Ont. Canada. L8N 3Z5.

Low concentrations of adenine and guanine nucleosides and nucleotides, when added to the medium of serum-deprived quiescent astroblasts or human astrocytoma cells, stimulated cell proliferation in a dose-dependent fashion. We used agonists and antagonists specific for various types of cell-surface adenosine and purine receptors to determine which receptors were involved. Adenosine had two peaks of activity, at ~100 pM and at ~7.5μM. Guanosine and inosine stimulated cell proliferaton more than adenosine in μM concentrations, but were inactive at pM concentrations. Activation of astroblast A2 receptors both stimulated cell division and increased intracellular cAMP.

Purine nucleotides stimulated astroblast proliferation in μM concentrations. However, α,β-methylene ATP, a preferential P2X agonist, maximally stimulated proliferation at 3.5nM. 2-methylthio ATP, a preferential P2Y agonist, had peak activity at 30pM. This implies that purine nucleotides stimulate astrocyte mitosis through a P2Y receptor.

Purine nucleosides and nucleotides are released in high concentrations in the CNS during neurotransmission, hypoxia and cell death. Our data imply that this may be sufficient to stimulate proliferation of various types of adjacent cells. (Supported by a grant from the Ontario March of Dimes)

413.22

NERVE GROWTH FACTOR AND GANGLIOSIDE REGULATED RELEASE OF FIBRONECTIN-LIKE AND J1/TENASCIN GLYCOPROTEINS BY C6 GLIOMA. E. Yavin. Dept. Neurobiology, Weizmann Institute of Science, Rehovot, Israel

C6 rat glioma cells incubated in serum-free medium with D-[14C]glucosamine secrete upon stimulation with nerve growth factor (NGF) or monosialogangliosides (MSG) a number of glycoproteins (Gp) the most prominent of which being a 270, 220, and a 69 kDa Gp. Several growth factors, hormones and phorbol ester as well as disialo- or trisialogangliosides did not stimulate secretion. Western blot analysis of the conditioned medium supplemented with NGF or MSG identified fibronectin and J1/tenascin each migrating as one distinct band of a molecular weight of approximately 220 kDa. Antiserum to NGF prevented NGF-stimulated release but it also blocked MSG-evoked release. Significant levels of the 220 kDa Gp were detected after pulse labeling with [^{35}S] methionine in the presence of NGF; after 15 min incubation immunoprecipitable radioactively labeled J1/tenascin was noticed. Tunicamycin inhibited most profoundly release of the 220 kDa Gp labeled either by D-[^{14}C] glucosamine or [^{35}S] methionine.

These results extend the range of neurotrophic properties attributed to NGF to cells of glial origin and suggest that NGF regulates secretion of extracellular matrix proteins. The data also suggest that MSG stimulation of fibronectin and J1/tenascin secretion is presumably mediated by an NGF or NGF-like molecule also secreted by the C6 glioma cells.

413.23

HEART CELL CHOLINERGIC NEURONAL DIFFERENTIATION FACTOR (LEUKEMIA INHIBITORY FACTOR) CAN REVERSIBLY REGULATE NEUROTRANSMITTER / PEPTIDE PHENOTYPE IN SEVERAL TYPES OF CULTURED PERIPHERAL NEURONS. Hiroyuki Nawa*, Tetsuo Yamamori, Thu Le, Donald Metcalf ** and Paul H. Patterson. Biol. Div. 216-76, Caltech, Pasadena, CA 91125; *Inst. for Immunol., Fac. of Med., Kyoto Univ., Kyoto 606 Japan; ** Walter and Eliza Hall Inst. Med. Res., Royal Melbourne Hospital, Parkville, Victoria 3050, Australia.

The various types of neural crest-derived peripheral neurons can be characterized by their distinctive combinations of neurotransmitters and neuropeptides. These phenotypes are plastic and respond to environmental cues *in vitro* and *in vivo*. The cholinergic neuronal differentiation factor (CDF) from cultured heart cells is identical to leukemia inhibitory factor (LIF), and recombinant LIF (rLIF) from *E. coli* can induce cholinergic differentiation in cultured rat sympathetic neurons (Yamamori et al., Science 246, 1412, 1989). We find that rLIF also induces the expression of substance P, somatostatin and vasoactive intestinal polypeptide, while reducing the expression of neuropeptide Y in a dose-dependent manner in the sympathetic neurons. The alterations in peptides are also observed at the mRNA level. The changes in peptide expression are reversed upon removal of CDF from the neurons, demonstrating that the factor is required for maintenance of the newly induced phenotypes. Dorsal root ganglion neurons also respond to rLIF, but with different phenotypic changes, suggesting that CDF/LIF could have widespread effects in the nervous system.

413.24

DIFFUSIBLE FACTOR(S) OF RETINAL PIGMENT EPITHELIAL CELLS MEDIATES SURVIVAL OF PHOTORECEPTOR CELLS IN A DYSTROPHIC MODEL: IN VIVO AND IN VITRO EVIDENCE. H.J. Sheedlo, L. Li and J.E. Turner, Depts of Neurobiol. and Anat. and Ophthalmol., Bowman Gray Sch. of Med., Winston-Salem, NC

Transplants of normal RPE cells in retinas of RCS dystrophic rats has been shown to arrest photoreceptor cell (PRC) loss, even lateral to the RPE transplant, suggesting PRC rescue may be affected by an RPE cell diffusible trophic factor(s). In one study, normal RPE cells were transplanted into the subchoroid of 26 day-old RCS dystrophic rats, separated from the PRCs by Bruch's membrane. Seven weeks later, PRC rescue was detected under and lateral to the RPE cell transplant. In a second study, normal RPE cells were encased in perm-selective hollow fibers (slow release mechanism) and placed in cultures of PRCs, isolated from day 2 Long Evans rats, and grown in a defined medium, lacking serum. Under these conditions, PRCs survived for at least a week, while in control fibers, lacking RPE cells, PRC survival was not observed. In addition, PRC cultures were supplemented with several growth factors. Only epidermal growth factor affected significant cell survival. These studies provide strong evidence that RPE cells secrete a factor(s) which affects PRC rescue and survival. Results of the slow release mechanism and vitreous injections in RCS dystrophic rats will be reported. (Fight for Sight, Inc. and NEI 04337)

413.25

TRANSFORMING GROWTH FACTOR-ALPHA (TGF-ALPHA) IMMUNOREACTIVITY IN ADULT RAT BRAIN. J. Lakshmanan, E. Salido*, R. Lam*, L. Krummen*, A. Reviczky* and D.A. Fisher*. Depts. of Pediatrics, Pathology and Endocrinology, Harbor-UCLA Medical Center, Torrance, CA 90509.

Evidence for the existence of TGF-alpha prepro mRNA in rodent brain has been reported (J Neurosci 8:1901, 1988) but limited information is available on the distribution of TGF-alpha in brain. Here we report TGF-alpha levels in rat brain as measured by a specific RIA developed in our laboratory. Chemically synthesized rat TGF-alpha was used for antisera generation, iodination and reference standard. The RIA is sensitive to 8 pg/tube. Whole brain, cerebral cortex, cerebellum, and midbrain of adult rats were homogenized in 50mM phosphate buffered saline (pH 7.4) and centrifuged at 100,000 x g for 60 minutes. The supernatants were used for quantification of TGF-alpha and protein contents. TGF-alpha concentration is expressed as pg/mg protein (values are mean ± SD). Whole Brain: 47±8; Cerebral Cortex: 72±16; Cerebellum: 162±18; Midbrain: 180±29. Conclusion: TGF-alpha is present in adult rat brain. Its concentration varies with brain region.

413.26

EXPRESSION OF ACIDIC-FIBROBLAST GROWTH FACTOR (a-FGF) mRNA IN DEVELOPING AND ADULT RAT BRAIN. B.J. Wilcox and J.R. Unnerstall, Department of Neurology and The Alzheimer Center, Case Western Reserve University School of Medicine, Cleveland, OH 44106.

Using a 36mer oligonucleotide probe corresponding to aminoacids 26-37 of bovine aFGF, we have localized mRNA for aFGF in developing and adult rat brain by *in situ* hybridization histochemistry. Nine time points in brain development from embryonic day 17 (E17) to post-natal day 21 (P21) were selected. Results of hybridization of [^{35}S]probe to slide-mounted parasagittal sections of brain tissue from earlier time points (E17-E20) showed generalized labeling throughout the brain with enrichments found in the developing cortex. Labeling was distinctly localized to the ventricular zone and cortical plate. At later time points (P1-P21), a distinct pattern of labeling to the pyramidal cell layer of the hippocampus was observed. During the second post-natal week, discrete labeling also appeared in the developing granule cell layer of the dentate gyrus. Examination of autoradiograms under high magnification confirmed the localization of grains to neurons in the hippocampus and cerebral cortex. The appearance of aFGF mRNA in the hippocampal formation and dentate gyrus corresponds with the maturation and migration of pyramidal and granule cells to their adult position. This pattern of labeling persisted into adulthood, although at a lower level of expression. Labeled neurons were also seen in laminae II, III, and VI of the cerebral cortex, granule cells of the cerebellum and other discrete nuclei. No labeling was seen when serial sections were hybridized with a corresponding sense probe. Northern blot analysis revealed a single band which agrees with the reported size for aFGF message in brain. These data indicate that aFGF is expressed in specific neuronal populations in the developing and mature CNS and suggest that this neurotrophic factor plays a significant role in CNS development and in maintenance of neuronal plasticity in adult brain.

414.1

PHYSIOLOGICAL EVIDENCE FOR FUSION OF SEVERED MYELINATED AXONS. T. L. Krause, R. E. Marquis, and G. D Bittner. Dept. of Zool., Coll. of Pharm., and Inst. for Neurosci., U. Texas, Austin, TX 78712.

We have recently reported the ability to morphologically fuse the severed halves of an invertebrate myelinated giant axon, the medial giant axon (MGA) of *Lumbricus terrestris* (Krause and Bittner, PNAS 87: 1471-1475). This morphological fusion is achieved in a few minutes using polyethylene glycol (PEG) in reduced-calcium hypotonic salines. Rates of morphological fusion were as high as 80-100% in some trials using this technique. We have extended this former work to evaluate both form and function of these fused axons.

We now report the ability to establish action potential conduction through the fusion site of formerly severed axons. Using extracellular and intracellular recording techniques, we have found through conduction in approximately 20% of fused axons in some trials. Further, we report that fused axons can retain morphological and physiological integrity after 16-26 hours, although physiological integrity is most often present when measured several hours after incubation. Finally, we have extended our study of conditions most conducive to establish high fusion efficiency and are attempting to repair severed axons *in vivo* using this fusion technology.

This research was made possible by a Texas Advanced Technology Project Grant (#194) and a National Science Foundation Grant (ECS 8915178) to G.D.B.

414.2

EPENDYMAL/GLIAL CELL PRODUCTION ALONG THE RETINOTECTAL TRACT PRECEDES OPTIC NERVE REGENERATION IN THE SALAMANDER. I. Gordon,* S.B. Simpson,# Jr., and B.M. Davis. Dept. of Anatomy & Neurobiology, *Univ. of Kentucky, Chandler Medical Center, Lexington, KY 40536; #Dept. of Biological Science, Univ. of Illinois, Chicago, 60680.

Our experiments on regenerating salamander spinal cord revealed that large numbers of ependymal cells along the path or regenerating axons incorporate 3H-thymidine during the initial phases of axon regeneration. To test the generalities of these results salamander optic nerves were crushed unilaterally followed by 3H-thymidine injections 0-14d or 0-21d post-crush (2-4μCi/gr, every other day). At the completion of the injection schedule the entire animal was processed for autoradiography. This allowed us to examine the entire course of the retinotectal projection. In all cases, large numbers of labeled glial cells were seen ipsilaterally along the entire length of the optic nerve. Labeled ependymal cells were seen bilaterally in the diencephalon, particularly in ventral portions. In some cases, large numbers of labeled ependymal cells were also seen in the contralateral mesencephalon. Labeled neurons were not identified in any of these cases. HRP injections showed that retinal axons had not reach the brain by 21 days post-crush. These results suggest that ependymal cell production is induced by damaged axons and confirm the observations of Gaze and Watson (1965) and Stevenson and Yoon (1978). It should be noted that the time course of the presumptive burst of glial/ependymal cell mitosis occurs within the first two weeks post-crush and could be the event underlying enhanced regeneration seen following a "conditioning lesion". Supported by NS25617 to BMD.

414.3

BEHAVIORAL RECOVERY FOLLOWING SPINAL TRANSECTION OCCURS VIA FRANK AXONAL REGENERATION SUPPORTED BY DIVIDING GLIAL CELLS ALONG THE PATH OF THE REGENERATING PROJECTIONS. L. K. Garner, D.R. Liebich,* S.B. Simpson, Jr.,# and B.M. Davis. Dept. of Anatomy & Neurobiology, *Univ. of Kentucky, Chandler Medical Center, Lexington, KY 40536; #Dept. of Biological Science, Univ. of Illinois, Chicago, 60680.

We tested the following hypothesis: CNS regeneration in the salamander does not require neurogenesis, but does require the production of new ependymal and/or glial cells along the length of the regenerating projections. HRP was applied to complete thoracic transections to label the somata of neurons whose axons projected past the transection site. After behavioral recovery (60-90d), Fast Blue was injected into the spinal cord 1.0 cm caudal to the first lesion. In regenerated salamanders the percentage of Fast Blue labeled supraspinal neurons that also contained HRP ranged from 6-23% (x=18%; n=7). In control experiments in which both markers were applied at the same time, the maximum number of doubled labeled cells was 27% (ranging from 3-27%; x= 15%; n=5). This suggests that most of the axons that grew through the transection arose from axotomized neurons. 3H-thymidine injections during the regeneration period indicated a burst of ependymal/glial cell production throughout the spinal cord. Within 5 mm of the lesion site, over 70% of the ependymal cells were thymidine labeled. In brachial and lumbar spinal cord the number of labeled cells was significantly greater than controls. These results suggest that damaged CNS axons induce glial cell production and that the new cells support frank axonal regeneration. Supported by NS25617 to BMD.

414.4

AXOSOMATIC SYNAPSES INCREASE ON LOCAL SPINAL MOTONEURONS DURING TAIL REGENERATION: DOES SYNAPSE FORMATION PREVENT AXONAL REGENERATION? M.T. Duffy,* B.M. Davis, and S.B. Simpson, Jr. Biological Sciences, University of Illinois, Chicago, 60680; #Anatomy & Neurobiology, Univ. of Kentucky, Chandler Medical Center, Lexington, KY, 40536.

In our previous studies we found that the number of supraspinal axons projecting to the tail increases by 74% during tail regeneration, but only a small fraction of these axons (<4%) enter the regenerated spinal cord (Duffy et al., 1990). We suggest that this may be the result of "synaptic capture" (Bernstein et al., 1978) in which regrowing axons make synapses on denervated targets rostral to the transection, aborting further regeneration. To examine this hypothesis we used morphometric analysis of EM photomontages to test for changes in synaptic distribution on motoneurons rostral to regenerating tail spinal cord.

Examination of lamina IX neurons (presumptive motoneurons) revealed the following properties: 1) Neurons rostral to regenerated tails (n=6) are larger in both circumference and area compare to non-regenerates (n=20) (79.7 μm vs 51.6 μm; 291.9 μm2 vs 124.0 μm2), 2) axosomatic contacts cover a greater percentage of the motoneuron soma following regeneration (32.25% vs 20.8%), and 3) this increased innervation is accomplished by more synaptic boutons rather than larger boutons (21 boutons/cell (avg length = 1.27 μm) vs 9 boutons/cell (avg length = 1.34 μm)). This result suggests that increased synaptogenic activity in the spinal cord immediately rostral to the junction of normal and regenerating spinal cord could be an important mechanism in the inhibition of CNS axonal regeneration.

414.5

H^3-GABA UPTAKE BY CEREBROSPINAL FLUID-CONTACTING NEURONS IN THE REGENERATED SPINAL CORD OF THE LIZARD. L. Alibardi*, J. Gibbons* and S.B. Simpson, Jr. Dept. of Biol. Sci. and the Comm. on Neurosci., Univ. of Ill. at Chicago, Chicago, IL 60680.

The regenerated spinal cords of several lizards have been reported to contain cerebrospinal fluid-contacting neurons (CSFCNs) (Alibardi, L. and V.B. Meyer-Rochow, New Zealand J. Zool., 15:535, 1988). We are attempting to characterize these neurons with respect to neurotransmitter type and their connectivity with the normal spinal cord and brain. We have recently confirmed the presence of CSFCNs in both the normal and the regenerated cord of the lizards Anolis and Scincella. The CSFCNs are probably the only neuronal elements to differentiate in the regenerated tail cord. Lizards injected with H^3-GABA (15-25 μCi/animal) demonstrate uptake by most, but not all, of the CSFCNs. Uptake was demonstrated at both light and TEM levels. Surrounding connective tissue cells and ependyma cells did not take up the H^3-GABA. Not all CSFCNs in a given preparation concentrated the H^3-GABA. This could be due either to their being at different stages of differentiation or to the existence of two different populations of CSFCNs, based on transmitter type. Using retrograde transport of horseradish peroxidase, we have also demonstrated that many of the CSFCNs in the regenerated tail cord send axons rostrally into the normal tail cord.

414.6

REGENERATION OF CENTRIFUGAL FIBERS TO THE RETINA IN CICHLID FISH. Anne C. Rusoff. Dept. of Biology, Montana State University, Bozeman, MT 59717.

The optic nerve of cichlid fish contains two populations of axons: the axons of retinal ganglion cells and axons from cells in the diencephalon and telencephalon that project into the retina (centrifugal fibers). The axons of retinal ganglion cells in fish are noted for their ability to regenerate. One factor often implicated in this ability is the continued addition of ganglion cells into adulthood. In contrast all of the centrifugally projecting cells in the telencephalon and most of those in the diencephalon are born early. This basic difference between the two populations of axons in the optic nerve allows one to determine if mitotic activity within the cell population is critical for regenerative ability. Previously I reported that FMRFamide + centrifugal fibers did not regenerate (Soc. Neurosci. Abstr. 14: 657 (1988)). Recently I have attempted to confirm this result using HRP placed either distal or proximal to the crush. When HRP was placed on the retinal side of the crush, cell bodies that had regenerated axons across the crush were labelled both in the diencephalon and telencephalon. When HRP was placed central to the crush, axons that had regenerated across the crush were visible in the retina. Therefore, centrifugal fibers are capable of regenerating even though they come from a cell population that has ceased dividing. (EY06495.)

414.7

REGENERATION OF BRAINSTEM-SPINAL AXONS AFTER SPINAL CORD TRANSECTION IN EMBRYONIC CHICKEN. S.J. Hasan, H.S. Keirstead* and J.D. Steeves. Departments of Anatomy and Zoology, University of British Columbia, Vancouver, B.C., V6T 2A9.

In the embryonic chicken, brainstem-spinal pathways to the lumbar cord complete their projections around embryonic day (E) 11 of the 21 day developmental period. Recently it has also been shown that brainstem-spinal tracts in the embryonic chicken maintain their capacity for anatomical and functional repair after a complete thoracic spinal cord transection prior to E13. This repair could be attributed either to projections from late developing unlesioned neurons or to regeneration of previously axotomized brainstem-spinal pathways. In this study, double labelling was used to distinguish between these two possibilities. On E8-11, the mid-thoracic spinal cord was injected with the first fluorescent tracing dye. One to two days later the mid-thoracic spinal cord was completely transected. After an additional 5-8 days, the second fluorescent tracing dye was injected into the cord, caudal to the site of transection. Two days later (on E17-20), the CNS was fixed, frozen sectioned and the brainstem and spinal cord tissue sections were viewed with epifluorescence microscopy.

A significant number of double labelled brainstem-spinal neurons were observed following a thoracic cord transection as late as E11-12. Each brainstem also contained a small number of cell bodies labelled with either the first or second fluorescent tracer alone (ie. single labelled). The double labelled brainstem-spinal neurons suggest that regeneration of transected projections contributes to the functional repair of spinal cord injuries in embryonic chicken. (Supported by the MRC of Canada and the Rick Hansen Man in Motion Legacy Fund.)

414.8

THE DEVELOPMENT AND REGENERATION OF THE SYMPATHETIC INNERVATION OF THE RAT TAIL ARTERY. C. R. Anderson* and Elspeth M. McLachlan. Department of Physiology and Pharmacology, University of Queensland, Queensland, 4072, Australia.

The sympathetic innervation of the main caudal artery of Wistar rats was examined using the SPG technique for catecholamine fluorescence, and immunohistochemical localisation of tyrosine hydroxylase (TH) and neuropeptide Y (NPY) during normal development and during regeneration following nerve lesions.

At birth, the tail was essentially devoid of sympathetic fibres. By 3d postnatal, small paravascular bundles extended along the proximal caudal artery and the first perivascular fibres were present. By 10d, sympathetic fibres were present over the media of the entire length of the caudal artery but the density of fibres found in the adult caudal artery was not achieved until 45d. At all earlier stages there was a proximo-distal gradient of maturation with a lag of about 7-10d in the most distal regions. Noradrenaline, (as visualised with catecholamine fluorescence), and TH and NPY were all present around the caudal artery from the earliest times that nerve fibres could be detected in the tail.

When the tail of 21d old rats was denervated by freezing all four collector nerves at the level of Co7-8 vertebrae, sympathetic fibres around the caudal artery degenerated from 2-3 cm below the level of the lesion. Within10d following denervation, sympathetic fibres began to reappear around the caudal artery,extending proximo-distally at about 2 mm/day. However, after 50d regenerated fibres were rarely found in the distal 30% of the artery, and this persisted as long as120d after denervation. In contrast, the density of regenerated terminals in proximal parts of the caudal artery was only slightly lower than that at equivalent levels of control arteries of the same age. The functional properties of the regenerating fibres are currently under study.

414.9

CHANGES IN SPINAL REFLEXES OF THE RAT INDUCED BY NEONATAL PERIPHERAL NERVE INJURY. B.S. Chung, K. Sheen, J.W. Leem and J.M. Chung. Marine Biomed. Inst., Depts. of Anat. & Neurosci. and of Physiol. & Biophys., Univ. Texas Med. Br., Galveston, TX, 77550.

To investigate the effect of neonatal peripheral nerve injury on the organization of the spinal cord, we examined spinal reflexes of adult rats (Sprague-Dawley) in which the left sciatic nerve had been cut during the neonatal stage.

After decerebration at the precollicular level under a gaseous anesthesia of O_2, N_2O and halothane, a laminectomy was performed at the L1-L6 segments. Electrical stimulation was applied to the L5 spinal cord and evoked compound action potentials were recorded in the ventral root of the same segment.

Based on latencies, multiple components of the evoked potentials could be identified: an early monosynaptic reflex and a delayed polysynaptic propriospinal reflex. In addition, spinobulbospinal reflexes could be identified as judged by the disappearance after spinal cord transection at the C1 level. The sizes of propriospinal and spinobulbospinal reflexes were significantly larger on the lesioned side than on the control side.

These results indicate that neonatal peripheral nerve injury induces alterations in the organization of the spinal cord, suggesting functional plasticity in the spinal cord. (Supported by NIH grants NS21266 and NS11255 and a grant from Bristol-Myers Co.)

414.10

AXONAL REGENERATION AFTER CHRONIC SPINAL CORD INJURY. J. D. Houle Dept. of Anatomy, Univ. of Arkansas for Medical Sciences, Little Rock, AR 72205.

The purpose of this study was to determine if neurons retain the ability to regenerate their axonal process for a prolonged period after injury to the spinal cord. True Blue was injected (TB) into one side of the adult rat lumbar spinal cord to label neurons whose axons course through this region. Seven days later these axons were injured by aspiration of the injection site, creating a hemisection cavity. Four weeks later, scar tissue was removed prior to grafting 1 cm long segments of autologous peripheral nerve (PN) to the rostral and caudal surfaces of the lesion cavity. Thirty days later, the distal tip of each of the PN grafts was exposed to Nuclear Yellow (NY) to label neurons that had grown an axon into the graft. Neurons containing both TB and NY were deemed capable of axonal regeneration while in a chronically injured state.

There were no TB/NY neurons within the brain, however, numerous dual labeled neurons were identified within Laminae IV through X (excluding IX) ipsilateral and Laminae VI and VII contralateral to the lesion site, with most cells located within 10mm of the lesion. Within individual lumbar dorsal root ganglia nearly 50% of the neurons labeled with NY also contained TB (range 24-74%). This population of regenerating neurons ($\bar{X}$=48, range of 10-189 per ganglion) had a smaller mean cell area (955 um^2) compared to those with TB only (1110 um^2). These results indicate that in a chronic spinal cord injury condition certain neurons have the capacity for axonal regeneration long after their initial response to injury. Supported by NIH Grant NS-26380.

414.11

REGENERATION OF HYPOTHALAMIC NEUROSECRETORY NEURONS INTO THE THIRD CEREBRAL VENTRICULAR LUMEN FOLLOWING HYPOPHYSECTOMY--AN ELECTRON MICROSCOPIC IMMUNOGOLD STUDY. Wutian Wu and David E. Scott, Dept. of Anatomy & Neurobiology, Eastern VA Med. School, Norfolk, VA 23501

The present investigation focusses upon the reorganization and regeneration of neuropeptide containing neurites into the third cerebral ventricle following hypophysectomy. Six male Spraque-Dawley rats were hypophysectomized 4 weeks before harvesting the brains. Hypothalamic tissue was prepared for immunogold staining. Primary antisera against arginine vasopressin (AVP), Oxytocin (OXT), and Tyrosine hydroxylase (TH) were applied with the "on-grid" immunomarking technique which utilizes secondary antibody-coated colloidal gold probes. Most of the neurites in the ventricular lumen were AVP positive. Gold particles were found within neurosecretory vesicles. Some OXT positive vesicles and AVP positive vesicles were found to be located in the same terminal. Both AVP and OXT positive neurites freely terminate within the third ventricular lumen. No TH positive terminals were observed within the ventricles, although TH neurons in hypothalamus also project to the posterior pituitary. The results of the present study suggest that neurosecretory neurons are able to regenerate their neurites and regrow them into the cerebral ventricular lumen after axotomy. Hence, the CSF may serve as a functional terminus for release of neuropeptide hormones. Supported by NSF grant BNS 8709687.

415.1

DIFFERENTIAL EXPRESSION OF AN ACETYLCHOLINE RECEPTOR PROMOTER-LacZ TRANSGENE IN MUSCLE NUCLEI. J.P. Merlie, and J.R. Sanes. Depts. Pharmacol. and Anat. & Neurobiol., Washington University Med. Sch., St. Louis, MO 63110.

Acetylcholine receptor subunit mRNAs are more abundant in innervated than denervated muscles and in synaptic compared to extrasynaptic areas of muscle fibers (Merlie et al., J. Cell Biol., 1984; Merlie and Sanes, Nature, 1985). To study the transcriptional basis of these nerve-dependent phenomena we produced transgenic mice containing an AChR α-subunit promoter fused to the coding sequences of chloramphenicol acetyltransferase (CAT) or β-galactosidase (lacZ). In mice transgenic for an α-promoter-CAT fusion, CAT expression was both muscle specific and increased 100X by denervation (Merlie and Kornhauser, Neuron, 1989). To investigate transcriptional differences among and within muscle fibers, we prepared transgenic mice in which the α-promoter fragment was fused to a lacZ gene modified by incorporation of the SV40 large T antigen nuclear transport signal. As determined histochemically, the distribution of lacZ in 3 lines of αnLacZ mice was muscle-specific. However, αnLacZ mice exhibited pronounced variation among muscles; e.g., tibialis anterior stained far more intensely than soleus. Surprisingly, however, αnLacZ expression was not affected by denervation. Furthermore, nuclei within single myotubes were differentially stained: some nuclei were unstained and synaptic nuclei were often more intensely stained than extrasynaptic nuclei. Although measurements of lacZ RNA will be needed to rule out posttranscriptional effects, we suspect that an activity-dependent regulatory element within the α-subunit promoter was suppressed by fusion to nLacZ. Thus, muscle-specific and activity-dependent regulation are dissociable. Furthermore, the gene fusion is apparently recognized and expressed differently in different muscles, presumably reflecting unique transcriptional potentials of each muscle. (Support: NIH)

415.2

C2 MUSCLE CELLS EXPRESS AGRIN BINDING MOLECULES. M.A. Nastuk[1], H. Gordon[2] and J.R. Fallon[1]. [1]Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545. [2]Dept. of Physiology, UCSF, San Francisco, CA 94143.

Agrin, a protein isolated from Torpedo electric organ, induces clustering of acetylcholine receptors (AChRs) on cultured chick myotubes. To elucidate the mechanism of agrin's action, we are working to purify and characterize the molecule(s) to which agrin binds as it interacts with myofiber surfaces. We have chosen the C2 mouse muscle cell line as a source of agrin binding molecules. Here we confirm previous findings (Gordon & Hall, SNS Abstr. 15:1352) that Torpedo agrin induces AChR clustering on the surfaces of C2 myotubes. Agrin binding sites also become redistributed as part of this response. Clusters of agrin binding sites, revealed by immunofluorescent labeling of bound agrin, are colocalized with spontaneous as well as agrin-induced AChR aggregates. C2 myoblasts also express agrin binding sites. NRK fibroblasts, however, do not bind agrin. For C2 myoblasts as well as myotubes, agrin binding depends upon the presence of extracellular calcium; EGTA abolishes virtually all specific binding much as in chick myotubes (Fallon, this vol.). These results suggest that calcium is important in regulating the action of agrin. Furthermore, the calcium dependence of agrin binding may prove highly useful in subsequent affinity purification strategies. NIH F32 NSO8152 and HD 23924.

415.3

TORPEDO ACHRs EXPRESSED IN MOUSE FIBROBLASTS CLUSTER IN RESPONSE TO EXTRACELLULAR MATRIX AND NG108-15 CONDITIONED MEDIA. D.S. Hartman* and T. Claudio. Dept. Cell. & Molec. Physiology, Yale Univ., New Haven, CT 06510.

Torpedo acetylcholine receptors (AChRs) stably expressed in mouse fibroblast L cells form fully functional receptor-channels (*Science* **238**, 1688-94, 1987) and are distributed evenly on the cell surface of the fibroblast (*J. Physiol., Paris*, **84**, 1990). AChR surface distribution was determined by labelling briefly with a monoclonal antibody specific to the AChR α subunit (mAb22) followed by a second antibody conjugated to phycoerythrin, and visualized at 570nm with a Zeiss IM35 microscope. A variety of agents were able to induce AChR clusters in fibroblasts. Prolonged incubation of the cells with mAb35, a monoclonal antibody previously shown to induce antigenic modulation of cell surface AChRs, produces small (<2μM) AChR clusters followed by rapid internalization of the clusters. Incubation of the AChR-fibroblasts with *Torpedo* extracellular matrix (ECM) extract or conditioned media from an NG108-15 neuroblastoma-glioma cell line, agents which have been shown to induce AChR cluster formation in cultured muscle cells, also induce the formation of AChR clusters in the fibroblasts. These clusters are larger than antibody clusters, and range in size from 2-10μM in diameter. Clusters induced by ECM or NG108-15 conditioned media do not turnover rapidly and the total number of surface AChRs does not change. *Torpedo* AChRs expressed in mouse fibroblasts appear to respond to various clustering agents in a manner similar to that of mammalian AChR in muscle. This result suggests that at least these clustering factors induce AChR clustering via direct intermolecular interaction with the AChR molecule.

415.4

THE POSTSYNAPTIC 43K PROTEIN CLUSTERS MUSCLE NICOTINIC ACETYLCHOLINE RECEPTORS IN *XENOPUS* OOCYTES. P. B. Scotland+, C. W. Luetje#, J. Patrick#, and S. C. Froehner+. +Department of Biochemistry, Dartmouth Medical School, Hanover, NH 03756 and #Division of Neuroscience, Baylor College of Medicine, Houston, TX 77030.

Nicotinic acetylcholine receptors (AChR) are localized at high concentrations in the postsynaptic membrane of the neuromuscular junction. A peripheral membrane protein of M_r 43,000 (43K protein) is closely associated with AChR and has been proposed to anchor receptors at postsynaptic sites. We have used the *Xenopus* oocyte expression system to test the idea that 43K protein clusters AChR. Immunofluorescence of oocytes injected with RNA made from the cDNA of mouse muscle AChR subunits shows that the receptors are uniformly distributed in the surface membrane. Oocytes co-injected with AChR RNA and RNA encoding the mouse muscle 43K protein display AChR clusters of 0.5-1.5 microns in diameter. AChR clustering is not a consequence of increased receptor expression in the surface membrane as determined by measurements of acetylcholine-induced currents. Furthermore, expression of 43K protein does not alter the distribution of lectin binding sites, demonstrating that AChR clustering is not a result of non-specific aggregation of all membrane proteins. The 43K protein is co-localized with AChR in clusters when the two proteins are expressed together and forms clusters of similar size even in the absence of AChR. These results provide direct evidence that the 43K protein causes clustering of AChR and suggests that regulation of 43K protein clustering may be a key step in neuromuscular synaptogenesis.

415.5

MUSCLE-DERIVED AGRIN IS A COMPONENT OF THE EXTRACELLULAR MATRIX. E. Lieth[1], C.A Cardasis[2] and J.R. Fallon[1]. [1]Worc. Fnd. Exp. Biol., Shrewsbury MA 01545 and [2]UMASS Med. Sch., Worcester MA 01605.

Chick muscle-derived agrin (M-agrin) is associated with regions of high acetylcholine receptor (AChR) density in vivo and in vitro. Its antigenic relationship to Torpedo agrin implicates it as a possible synaptic organizing molecule. In vivo synaptic organizing activity resides in the basal lamina. Here we show that M-agrin is detectable by immunofluorescence on unpermeabilized cells in culture, and is not extractable in 2mM EGTA or 0.1% Triton X-100. It is solubilized in 0.2M bicarbonate pH 9, a buffer which also extracts agrin from Torpedo extracellular matrix. The pattern of M-agrin immunostaining in cultures is similar to that of laminin, but its distribution is more restricted. Furthermore, EM-immunocytochemistry demonstrates that M-agrin is a myotube basal lamina component. These results indicate that muscle secretes agrin-like molecules that become stably associated with the synaptic basal lamina. PHS grant HD 23924 to JRF and MDA fellowship to EL.

415.6

NERVE- AND AGRIN-INDUCED ACETYLCHOLINE (ACh) RECEPTOR CLUSTERING IN *XENOPUS* MULTINUCLEATE MYOTUBES. M. Saito and Y. Kidokoro. Jerry Lewis Center, UCLA School of Medicine, L.A., CA 90024

Agrin, which was extracted from the basement membrane of the electric organ of *Torpedo californica*, caused ACh receptor clustering in *Xenopus* multinucleate myotubes in culture. We tested a hypothesis that agrin released by the motor nerve terminal binds to its receptor in the basement membrane, and subsequently activates the receptor aggregating mechanism in the muscle cell.

In *Xenopus* multinucleate myotubes both nerve- and agrin induced receptor clustering were, at least partly, due to lateral migration of receptors in the membrane. Concanavalin A blocked both nerve- and agrin-induced clustering presumably by immobilizing ACh receptors. Heparin applied in the culture median partially blocked nerve-induced receptor aggregation and totally inhibited agrin-induced clustering. In contrast, a similar proteoglycan, chondroitin sulphate type A, affected neither of these receptor aggregating processes. Agrin in the culture medium at saturating concentrations partially blocked nerve-induced receptor accumulation.

Our observations are in accord with the hypothesis stated above.

415.7

INDUCTION OF ACETYLCHOLINE RECEPTOR CLUSTERING BY LOCALIZED APPLICATION OF SEVERAL GROWTH FACTORS IN XENOPUS MUSCLE CELLS. L.P. Baker and H.B. Peng. Curr. in Neurobiology & Dept. of Cell Biology and Anatomy, Univ. of North Carolina, Chapel Hill, NC 27599.

The abstract by Peng and Baker (this volume) discusses the localized application of bFGF to *Xenopus* muscle cells via beads, and its ability to induce the development of postsynaptic specializations. This work shows that in addition to bFGF, insulin and IGF-1 are also able to induce clustering of acetylcholine receptors (AchRs). Specific receptors for these ligands are known to be present on muscle cells. EGF, two isoforms of PDGF, and NGF-ß are unable to induce clustering. Many effects of growth factors are mediated through receptor-associated tyrosine kinase activity. In order to demonstrate the involvement of this activity in AchR clustering, a tyrosine kinase inhibitor, tyrphostin RG50864, was applied to the cells prior to bead application. AchR clustering was completely and reversibly blocked by 40 to 80μM RG50864. In addition, clustering induced by polycation-coated beads, as has been previously demonstrated by Peng, was inhibited by 40μM RG50864. Future work will be carried out to further characterize the specificity of cellular responses to tyrphostins, and to demonstrate tyrosine phosphorylation of growth factor receptors and their substrates in response to this localized application of growth factors. (Supported by NIH grant NS23583 and Muscular Dystrophy Association)

415.8

PARTIAL PURIFICATION OF AN ACETYLCHOLINE RECEPTOR AGGREGATING FACTOR FROM FETAL PIG BRAIN. A. Olek, N. Yu*, E. Dutton, and M. Melton. *Department of Zoology, University of* Maryland, College Park, MD 20742

It has been shown that developing neurons or neural tissue contain factors that either induce acetylcholine receptor (AChR) aggregation or stimulate the synthesis of AChR on aneural cultured myotubes. AGRIN, a highly purified factor obtained from Torpedo electric organ induces AChR aggregation (Nitkin et al., J. Cell Biol. 105:2471, 1987). ARIA (Usdin and Fischbach, J.Cell Biol. 103:493, 1986) and CGRP (New and Mudge, Nature, 323:809, 1986) increase the synthesis of AChR. While relatively crude extracts of mammalian neurons or neural tissues contain AChR aggregating activity, the factor has not been extensively purified.

We report the partial purification of a proteinaceous factor from fetal pig brain. This factor induces AChR aggregation in aneural cultures of rat myotubes within 4 hrs at 36° C. This factor has been purified approximately 5000 fold and is associated with a high (100-200 kd) molecular weight protein, active at nanomolar concentrations. The factor elutes from an anion exchange column with a salt concentration between 25 and 125 mM. The most purified fraction migrates on SDS-PAGE gels as 6 major bands from 74 to 170 kd and one band > 200 kd. Activity can be eluted from a small segment of non-denaturing gels containing only a few major protein bands. Protease digestion or incubation at 60° C for 30 min completely eliminates the activity of the aggregating factor.

415.9

NEURAL FACTOR-INDUCED ACETYLCHOLINE RECEPTOR AGGREGATION: A MODEL FOR LOCAL INDUCTION. M.A. Melton, A.J. Olek, and E.K. Dutton. Department of Zoology, University of Maryland, College Park, MD 20742.

During neuromuscular synapse formation in culture the neuron can induce the accumulation or aggregation of acetylcholine receptors (AChR) at the innervation site on myotubes. It has been proposed that the neuron transiently releases a factor that locally induces AChR aggregation. In support of this hypothesis, we have shown that local application of a partially purified mammalian AChR aggregating factor induces local AChR aggregation within 4 hrs on cultured rat myotubes at 36° C.

Primary rat myotube cultures were incubated in 0.3% Dispase to remove overlying fibroblasts and other non-muscle cells to produce experimentally accessible surfaces. Aggregates formed predominantly on the top surface of these myotubes in response to a 30 min bath applied pulse of a partially purified protein factor derived from fetal pig brain. Local application of the aggregating factor via micropipette to regions of the myotube surface for 30 min or less resulted in AChR aggregation largely restricted to the release site. Similar application of control solutions to the myotube surface had no effect. Further, preliminary studies indicate that this response was not associated with a detectable change in the membrane potential during the period of factor application.

415.10

THE *TORPEDO* POSTSYNAPTIC 280K PROTEIN IS DYSTROPHIN. S.C. Froehner#, M.H. Butler#, A.A. Murnane#, K. Douville#, and R. Sealock*. #Department of Biochemistry, Dartmouth Medical School, Hanover, NH 03756 and *Department of Physiology, University of North Carolina, Chapel Hill, NC 27599.

Purified postsynaptic membranes from *Torpedo* electric organ contain several peripheral membrane proteins that are thought to play a structural role at the synapse. One of these is a peripheral membrane protein of approximately M_r 280,000 (280K protein). Two monoclonal antibodies that recognize different epitopes on this protein were isolated. Immunogold labeling of electric organ with these mabs shows that the 280K protein is restricted to the cytoplasmic side of the innervated membrane in a distribution similar to the nicotinic receptor. By immunofluorescence, both anti-280K mabs stain muscle endplates very intensely and also show specific staining of extrasynaptic membrane. A protein of similar M_r in *Torpedo* postsynaptic membranes is specifically recognized on Western blots by antiserum to dystrophin. To examine the relationship between the 280K protein and *Torpedo* dystrophin, the former was purified by immunoaffinity chromatography. Purified 280K protein was recognized on Western blots by both anti-280K mabs and by anti-dystrophin but not by control antibodies. Furthermore, both anti-280K mabs stain normal mouse skeletal muscle, but not skeletal muscle from the *mdx* dystrophic mouse, which lacks dystrophin because of a mutation in the dystrophin gene. These results indicate that the 280K protein is dystrophin (or a closely related protein) and suggest that dystrophin may play an important role at the neuromuscular postsynaptic membrane.

415.11

EVIDENCE FOR ASSOCIATION OF THE POSTSYNAPTIC CYTOSKELETAL 58K PROTEIN WITH DYSTROPHIN. M.H. Butler, A.A. Murnane, and S.C. Froehner. Department of Biochemistry, Dartmouth Medical School, Hanover, NH 03756.

Several peripheral membrane proteins, including the M_r 58,000 (58K) and M_r 280,000 (280K) proteins are associated with the postsynaptic membrane of *Torpedo* electric organ and mammalian skeletal muscle. The 58K and 280K proteins are found at high concentrations on the cytoplasmic side of the neuromuscular postsynaptic membrane, but are also associated with the extrasynaptic sarcolemmal membrane. We recently identified the 280K protein as dystrophin or a closely related protein. Here we report evidence for the interaction of the 58K protein with dystrophin. Because of a mutation in the gene, dystrophin is absent from skeletal muscle of *mdx* mice. Immunofluorescence staining of *mdx* skeletal muscle by anti-58K mabs is severely reduced when compared to normal muscle. The reduction is selective for the 58K protein since staining of several other cytoskeletal proteins, such as vinculin, and a membrane protein (Na, K-ATPase) is normal in *mdx* muscle. Furthermore, preliminary experiments show that immunoprecipitation of Triton-solubilized *Torpedo* postsynaptic membranes with anti-58K mab also precipitates dystrophin. These results suggest that association of the 58K protein with the skeletal muscle plasma membrane requires the presence of dystrophin, possibly because the two proteins are present in a complex.

415.12

DIFFERENTIAL LABELING OF TERMINAL ARBORS OF MULTIPLE AXONS INNERVATING THE SAME TARGET CELL WITH DiI AND A NEW LIPOPHILIC TRACER, 4-Di-16-ASP. C.K. Chua* R.J. Balice-Gordon & J.W. Lichtman, Dept. Anatomy & Neurobiology, Wash. Univ. Sch. Med., St. Louis, MO 63110.

Competitive interactions between neurons vying for capture of the same target cells are believed to shape patterns of synaptic connections throughout the developing nervous system. Such interactions are poorly understood, in part because it is technically difficult to distinguish the terminals that persist from those that are eliminated. We have addressed this problem by differentially staining the terminals of competing axons using DiI, which fluoresces red (Honig and Hume, J. Cell Biol. 103: 171-187, 1986), and a new lipophilic compound, 4-Di-16-ASP (DiA; Mol. Probes; Soc. Nsci. Abs. 15: 163, 1989), which fluoresces green. DiA labels cell membranes and can be used as an antero- or retrograde tracer. In paraformaldehyde fixed tissue, DiA diffuses somewhat faster than DiI; importantly, however, these compounds stay segregated even when DiI and DiA labeled axons are interspersed. At embryonic neuromuscular synapses transiently multiply innervated by two different motor axons, one labeled with DiI and the other with DiA, each axon's terminals are similar to growth cones, having many filopodia and only 1-2 small terminal regions per axon. Surprisingly, both axons add discrete synaptic sites for a time, resulting in extensive intermingling of the two inputs. Over time, however, one axon acquires a majority of the synaptic territory, both by expansion of its territory and by the stepwise loss of synaptic sites by the other axon, which is finally resorbed into a retraction bulb proximal to the junction. We are presently studying how postsynaptic ACh receptor regions are occupied by competing axons during this period by labeling the same muscles with fluorescent alpha bungarotoxin.

415.13

NEUROMUSCULAR JUNCTIONS ON POLYNEURONALLY INNERVATED FIBERS SHOW INCREASED SYNAPTIC DEPRESSION. R. Dunia and A.A. Herrera, Dept. of Biological Science, University of Southern California, Los Angeles, CA 90089.

Intracellular recording revealed a growth-associated rearrangement in the innervation of the pectoral muscle of female *Xenopus*. Most fibers (90%) were innervated at two distant junctional sites. In small frogs (5-16 g), 60% of the fibers were innervated at both sites by the same motoneuron (A/A), while the remaining 40% received inputs from different motoneurons (A/B). In larger frogs (20-47 g), however, 93% of the fibers were A/A. We hypothesize that, as in developmental synapse elimination, competitive interactions in polyneuronally innervated fibers (A/B) result in synapse replacement and conversion to mononeuronal innervation (A/A).

To look for physiological correlates of competition, we recorded curare-blocked end-plate potentials (EPPs) from both junctions on single fibers and calculated the ratio of EPP sizes (smaller/larger) for each fiber. The mean ratio was 0.60±0.02 (sem) for A/A fibers, and 0.51± 0.05 for A/B fibers ($p>.05$). When the 50th EPP in a 10 Hz train was measured at the same junctions, the ratio increased in A/A fibers (0.66±0.03) and decreased in A/B fibers (0.44±.06). The difference was significant ($p<.005$), showing that synaptic depression was more pronounced in A/B fibers. These same junctions are being examined for morphological signs of synapse replacement.

415.14

INTERSTITIAL CELLS AT DEVELOPING NEUROMUSCULAR JUNCTIONS. E. A. Connor and R. A. Horowitz*, Univ. of Massachusetts. Amherst, MA 01003.

The structure and function of skeletal muscle depends on its innervation state. In innervated muscle, acetylcholine receptors and N-CAM are found at neuromuscular junctions. In non-innervated muscles, developing or denervated, these molecules are distributed throughout the muscle fiber membrane. The composition of the muscle connective tissue is also dependent on innervation . Interstitial cells accumulate in junctional regions of denervated muscle. In these same regions are found interstitial deposits of tenascin, fibronectin, and heparan sulfate proteoglycan. We asked whether similar changes in the cellular and molecular composition of connective tissue occur in developing muscles. Here we report that the distribution of interstitial cells in developing muscles resembles that in denervated muscles; interstitial cells are concentrated in junctional regions. Interstitial cell density was quantitated in cutaneous pectoris muscles of bullfrog larvae at stages XVI to metamorphosis, stage XXV. At stage XXV, interstitial cells were evenly distributed throughout the muscle. However, there was a gradient of interstitial cells in stage XVI muscles; junctional density of interstitial cells was 2.3 times that in extrajunctional regions. At later stages (XVII-XIX), the interstitial cell gradient was gradually reduced and was undetectable at stage XX. The interstitial cells had characteristic features of fibroblasts and resembled the accumulated interstitial cells in denervated muscle in position and morphology. The presence of an interstitial cell gradient in both developing and denervated muscles suggests that the interstitial cells or the matrix molecules that they produce play a significant role in synapse formation.

PROCESS OUTGROWTH, GROWTH CONES AND GUIDANCE MECHANISMS VIII

416.1

ASSOCIATION NEURONS OF EMBRYONIC SPINAL CORD EXPRESS THE CELL SURFACE GLYCOPROTEIN TAG-1. J.E.Vaughn, P.E.Phelps, M.Yamamoto and R.P.Barber. Division of Neurosciences, Beckman Res. Inst. of the City of Hope, Duarte, CA 91010.

There are two early-developing groups of dorsal horn interneurons in embryonic rat spinal cord: commissural (C) and association (A) cells that innervate contralateral medial and ipsilateral lateral motoneurons (LMNs), respectively. Dodd *et al.*(Neuron 1:105,1988) have shown that C neurons express the surface glycoprotein TAG-1 early in development, and we recently have found that this molecule is also expressed by A cells. On embryonic day 13 (E13), TAG-1 was expressed intensely on C axons, but some TAG-1-immunoreactivity (TAG-1-IR) was also observed on A fibers. By E14, both C and A cells expressed intense TAG-1-IR, with A fibers originating just ventrolateral to the source of C fibers in the dorsal intermediate zone. The A fibers coursed ventrally along the lateral margin of the intermediate zone and terminated adjacent to the dorsal-most LMNs, as defined by ChAT-IR. The origins of C and A fibers diverged progressively on E15 and E16. At these times, A fibers coursed along the entire lateral edge of the LMNs and penetrated medially among this nuclear group. By E17, TAG-1-IR was more intense on A than C fibers. Studies are in progress to determine if these TAG-1 fibers provide pathways for neuronal migration. *Supported by NIH grants NS18858 and NS25784.*

416.2

EXPRESSION OF A FASCICLIN-LIKE MOLECULE DURING NEURODEVELOPMENT IN THE COCKROACH. L.S. Wang* and J.L. Denburg. Dept. of Biology, The Univ. of Iowa, Iowa City, IA 52242.

The presence of molecules involved in specific fasciculation of axons during the development of the nervous system can be identified with monoclonal antibodies. One such Mab, DSS-8, was selected for study because it transiently binds to the nervous system of embryos of the cockroach, Periplaneta americana. Like previously described fasciclins the DSS-8 antigen was regionally expressed on the surface and on particular portions of subsets of growing axons in correlation with their pattern of selective fasciculation. However, in addition, DSS-8 labeled the first axons pioneering a specific subset of CNS axon pathways. In particular, the antigen was present on the first axons of the median fiber tract, the anterior commissure and the posterior commissure. The spatial distribution and temporal expression of the DSS-8 antigen during development suggest that it is playing a role in cell recognition occurring during specific axon fasciculation and in the reading of specific environmental cues during initial axon growth.

416.3

ANALYSIS AND PURIFICATION OF AXON FASCICLE SPECIFIC ANTIGENS IN THE LEECH K.M. Johansen, K.K. Erickson and J. Johansen. Department of Zoology, Iowa State Univ., Ames, IA 50011.

Two monoclonal antibodies, Ian 3-2 and Ian 4-2, label a small subpopulation of axon fascicles in the nerve roots and interganglionic connectives of the leech nervous system. The antigens are surface glycoproteins and on western blots Ian 3-2 recognizes three protein bands with molecular weights of 130, 105, and 90 kD, respectively; whereas Ian 4-2 recognizes a single 130 kD band (McKay et al., Science **222** : 788). Perturbation experiments with Fab fragments of Ian 3-2 have demonstrated that normal fascicle formation is disrupted by the antibody (Zipser et al., Neuron **3** : 621). The antigens recognized by these antibodies are therefore strong candidates for being molecules involved in selective fasciculation and neuronal recognition.

Immunoprecipitations with Ian 3-2 and Ian 4-2 have demonstrated that the two antibodies label the same 130 kD protein and thus recognize different epitopes on the same protein. These epitopes are expressed in at least 6 leech species from two different orders. This suggests that the epitopes are of functional importance since they have been conserved through leech divergence and evolution. Furthermore, using lentil-lectin affinity chromatography followed by immunoprecipitation, we have purified the antigens to homogeneity as judged by SDS-PAGE and silver staining of the gels. We estimate that the antigens constitute roughly 0.004% of total extractable CNS protein. Experiments are in progress to purify enough antigen for partial amino acid sequence determination and subsequent cloning of the gene. This work was supported by a Iowa State Biotechnology Grant and a Iowa State University Grant.

416.4

ASTROGLIAL DIFFERENTIATION, NOT "AGE", IS CRITICAL FOR NEURITE OUTGROWTH L-C. Wang, D.H. Baird, M.E. Hatten, and C.A. Mason. Dept. Pathology, Coll. Physicians and Surgeons, Columbia Univ., New York, N.Y. 10032

Current models of astrocyte differentiation stress a fixed sequence of steps, a timetable thought to be followed when glial cells are placed into tissue culture. This model predicts that glial support of neurite extension declines with the "age" of the glia, i.e., time in culture. An alternative view is that glial support of neurite growth involves a set of independently regulated events, some of which would be controlled by cell-cell interactions. To test these possibilities, purified mouse cerebellar astroglia were maintained *in vitro* for 3 days to 6 weeks in the absence of neurons, explants of pontine nuclei added, and neurite outgrowth quantitated 2 days later. Over time in culture, support of neurite extension declined, from a relatively robust level to negligible levels after 3-4 weeks. Since previous studies have shown that neurons induce the differentiation of glial cells (Hatten *J. Cell Biol.* 100:384, 1985), we tested the neurite promoting activity of glia kept in culture and induced to differentiate by the addition of neurons at different times (3 days to 6 weeks). When granule neurons were added at low density (0.1-0.5 x 10^6/ml) to glia and explants plated 2 days later, neurite growth from the explants was exuberant, regardless of the time glia spent *in vitro*. At this density in the absence of glia, cerebellar granule neurons have little or no effect on pontine neurite extension. We conclude that the differentiated properties of astroglia, here induced by co-culture with neurons, are critical for neurite extension, and that these properties can be modified independent of glial "age" in culture. Supported by NS21457 and NS16951.

416.5

GLIAL FIBER GEOMETRY ALONE IS NOT SUFFICIENT TO SUPPORT GRANULE NEURON MIGRATION. R.B. Fishman & M.E. Hatten. Ctr. for Neurobiology & Dept. of Pathology, Columbia University, Coll. of Physicians & Surgeons, N.Y., N.Y. 10032.

In vitro heterotypic recombination experiments show that cerebellar granule neurons are capable of migrating on hippocampal astroglia (Gasser & Hatten, PNAS, USA 1990, in press). Conservation of glial guidance across brain regions suggests that glia provide a generic, and possibly passive substrate for migration. To address this question, we examined whether *geometry* of the glial fiber alone is sufficient to support granule neuron migration.

Using an *in vitro* mouse cerebellar system, we tested whether granule neurons could migrate on a) glass fibers of similar geometry to glial fibers, and b) lightly fixed glial processes. Single glass fibers (~1-2um diameter) were obtained by vortexing Whatman glass fiber filters (GF/A). Fibers were coated with either polylysine, membrane from primary astroglia, or membrane from an astroglia tumor cell line, and subsequently co-cultured with purified cerebellar granule neurons obtained from postnatal day 4 mice.

Although granule neurons bound to glass fibers, and some neurons showed shape changes characteristic of migrating neurons, e.g. elongate profiles and "leading" process extension, neurons did not migrate on glass fibers. Furthermore, preliminary studies show that neurons cultured with lightly fixed (1% paraformaldehyde) glia bound to these glia but did not assume migrating profiles on them. These results suggest that geometry of the glial fiber alone is not sufficient to support migration, but rather migration requires an active interaction between neurons and living glia. Supported by NS15429.

416.6

CENTRAL NEURITE OUTGROWTH OVER RESTING AND REACTIVE ASTROCYTES P. Bovolenta, F. Wandosell* and M. Nieto-Sampedro. Neural Plasticity Lab., Cajal Institute, 28002 Madrid, Spain.

Protoplasmic astrocytes are a preferential substrate for neurite extension whereas, after CNS injury, fibrous astrocytes in the glial scar are regarded as a severe hindrance to axonal regeneration. To examine the cellular basis for this contradictory behavior, we have compared neurite outgrowth from explants of rat retina, septum and spinal cord cultured over purified astrocytes from newborn rat (10 to 30 days in culture); type 2 astrocytes; the same astrocytes treated with di-butyryl-cAMP or with phorbol ester; astrocytes grown in three-dimensions; membranes from injured parietal cortex and myelin. Neurites from the explants grew over all substrata, excepting myelin. Type 1 and type 2 astrocytes of various ages, flat polygonal astroblasts and astroblasts made to assume a star-shaped morphology with the help of diBcAMP, behaved similarly. The walls of a brain injury cavity is, 15 days postlesion, formed to a large extent by reactive astrocyte processes. Membranes prepared from these cells supported neurite outgrowth similarly to cultured astrocytes. In summary, regarding neurite outgrowth, we did not observe significant differences among the various kinds of astrocytes. We conclude that type 1, type 2 and reactive astrocytes are all good substrates. In the case of an open injury, the most likely way in which astrocytes hinder axon regeneration is by "misguiding" the regenerating sprouts away from their targets.
(Supported by a grant from the Spanish Science Research Council).

416.7

GROWTH OF RETINAL AXONS ON GOLDFISH OPTIC NERVE OLIGODENDROCYTES *IN VITRO* M.Bastmeyer, J.Vielmetter, G.Jeserich* and C.A.O.Stuermer, Friedrich-Miescher-Lab. der Max-Planck-Ges., Tübingen, FRG and *Univ.Osnabrück, FRG.

Mammalian oligodendrocytes inhibit the growth of neurites (Schwab and Caroni, 1988). Even retinal growth cones of goldfish collapse upon contact with these cells (Bastmeyer et al, 1988). Oligodendrocytes of regenerating fish optic nerves differ from mammalian oligodendrocytes.

1. Glial cells from fish optic nerves proliferate *in vitro* over weeks. After 2-3 weeks *in vitro* most of these cells express GFAP but also fish myelin glycoproteins detected by the monoclonal antibody (Mab) 6D2 (Jeserich et al, 1990) and the Mab O4-antigen found on mammalian oligodendrocytes (Sommer and Schachner, 1981).
2. Unlike mammalian oligodendrocytes, fish oligodendrocytes promote the growth of regenerating goldfish retinal axons *in vitro*. Both, regenerating fish retinal axons (Vielmetter and Stuermer, 1989) and the fish oligodendrocytes carry the Mab ϵ587-antigen, which has homologies to the mouse cell adhesion molecule L1. In mammals L1 is expressed on Schwann cells, but not on oligodendrocytes.

To test whether fish oligodendrocytes are growth permissive for neurites from other species, we cocultured embryonic chick retinal explants and goldfish glial cells. Chick retinal axons grew in high density on the surface of 6D2 positive goldfish oligodendrocytes. Thus, fish optic nerves through which retinal axons regenerate have oligodendrocytes that - at least *in vitro* - promote the growth of axons.

416.8

A METHOD FOR IDENTIFYING MOTONEURONS FROM INDIVIDUAL POOLS IN CULTURE. V. Boss and D. J. Wigston, Depts. of Biology and Physiology, Emory University, Atlanta, GA 30322.

We have developed a procedure for identifying axolotl motoneurons from distinct motor pools in cultured spinal explants. This will enable us to examine in culture the cellular mechanisms underlying the selective reinnervation of appropriate target muscles previously observed in vivo.

The lipophilic fluorescent dye 4-Di-10-ASP (Molecular Probes) was found to retrogradely label the cell bodies of motoneurons, as well as their neurites and growth cones which regenerated in culture. Motoneurons innervating hindlimb muscles of juvenile (6-8cm) axolotls were retrogradely labeled by injection of a 2% solution of 4-Di-10-ASP (in 95% ethanol) into spinal nerve 17. The nerve was crushed at the injection site to facilitate uptake. The dye was retrogradely transported and could be found within motoneuron cell bodies in 7 days and remained for at least several weeks. Therefore, after 7-20 days the ventral portions of lumbar spinal cord segments containing the labeled motoneuron cell bodies were removed and cut into small pieces. These explants were embedded in a collagen-laminin matrix and cultured at room temperature in a modified L15 medium, containing 10% fetal calf serum and either axolotl embryo extract or extract of denervated adult axolotl muscle. Following 4-6 days in culture, labeled motoneurons could be seen within the explants, extending brightly fluorescent processes away from the explants. Since growing processes as well as cell bodies can be labeled, interactions between neurites of identified cells and target tissues in culture can be examined. In addition, since another fluorescent dye, DiI, also retrogradely labels neurons and their growing processes in culture, and its fluorescence excitation spectrum is different from that of 4-Di-10-ASP, it should be possible to compare the interactions between neurons from 2 different motor pools and a given target tissue in the same culture.

416.9

INITIAL MOTOR AXON OUTGROWTH. D. Dehnbostel* & K. W. Tosney. Biology Dept., Univ. of Michigan, Ann Arbor, MI 48109.

1) What environmental features determine where motor axons exit from the spinal cord? 2) What interactions exterior to the cord mediate the patterned advance of axons? We are addressing these questions by assessing initial axon outgrowth in thin sections selected from serial, 25µm sections of stage 17 chick embryos prepared as in Tosney and Landmesser (J Histo Cyto 34: 953). 1) Growth cones accumulate at nascent exit points that are spatially predictable but without unique ultrastructural features. Growth cones displace neuroepithelial endfeet from the basal lamina, which appears to tear and be carried away by the first growth cones. Cells also exit the spinal cord, but do not precede growth cones. 2) Upon exiting the spinal cord, growth cones confront populations that provide differential environments for their advance. The posterior sclerotome and the ventral sclerotome in the anterior of a segment act as barriers; only the dorsal-anterior sclerotome acts as a pathway (Oakley and Tosney, 1990 NS Abstr.). Axons do have an opportunity to associate with both barrier and pathway tissues: axons exit in both the anterior and posterior of a segment and in each case traverse an adjacent vascularized region. Differences are seen only after contact with sclerotome. Axons in dorsal-anterior sclerotome ramify widely, extend directly dorsally and laterally, and initially form small fascicles. In contrast, axons that enter the more inhibitory environments cluster into larger fascicles and turn within a few microns. These differences in axonal trajectories and in neurite association support a substratum-preference mechanism of guidance, in which entry into a more inhibitory environment discourages advance and promotes closer association among neurites. Supported by NIH grant NS-21308.

416.10

EVIDENCE FOR THE DELINEATION OF AXON PATHWAYS BY INHIBITORY BOUNDARIES. R. A. Oakley and K. W. Tosney. Neurosci. Program & Biol. Dept., U. of Michigan, Ann Arbor 48109.

We previously demonstrated that peanut agglutinin (PNA) binds to three tissues adjacent to axon pathways in the chick embryo (the posterior sclerotome, girdle precursor, and perinotochordal mesenchyme; PNM) but not to the axon pathways themselves (Oakley and Tosney, NS Abstr. 347.7, 1988). We report here the distribution of PNA binding as detected with immunohistochemistry following three types of embryonic surgery. 1) Differential PNA binding patterns are retained even in the virtual absence of axons. Following unilateral neural tube deletion, axon pathways to the limb remain PNA-negative, including the dorsal-anterior sclerotome, the plexus region, and the hiatuses of the girdle which transmit axons to the limb. We conclude that *the differential pattern of PNA binding is independent of motor axon outgrowth.* 2) We find that axons turn to avoid the PNM at all stages of outgrowth following neural tube rotations that alter the initial direction of axon outgrowth so as to directly confront motor growth cones with the PNM. We conclude that *the dorsal anterior sclerotome is permissive and the PNM is relatively inhibitory for axon advance.* The PNM, like the posterior sclerotome and girdle precursor, thus acts as a barrier to axon advance. 3) In contrast, the PNM does not exhibit inhibitory function and does not differentially bind PNA following notochord deletion. *The inhibitory function of the PNM correlates with the expression of PNA binding epitopes.* We suggest that general axon pathways may be determined in part by relatively inhibitory characteristics of those tissues that express PNA binding epitopes.

Supported by NIH grants NS-21308 and NS-27634.

416.11

DEVELOPMENT OF THALAMOCORTICAL CONNECTIVITY IN VIVO AND IN VITRO. Zoltán Molnár* and Colin Blakemore. University Lab. of Physiology, Parks Road, Oxford OX1 3PT, U.K.

We are interested in the mechanisms responsible for the innervation of different cortical areas by particular nuclei of the thalamus. In organotypic co-cultures, any region of embryonic (E) 16/17 day rat thalamus will innervate any region of cortex (postnatal, P0-8). No positional preference was observed even when the thalamic explants were given a choice of different cortical targets. McConnell et al. (1989; *Science* 245:978) showed that subplate neurons pioneer the first axon pathway from the cerebral cortex to the thalamus in cat. We have determined the developmental time course of the establishment of these projections in rat from E13-E17. Tracing with fluorescent dyes from different cortical areas, we observed that these early corticofugal fibres have different projection targets in the diencephalon according to their cortical origin. By E16 the first corticopetal diencephalic fibres have reached their appropriate cortical areas using routes similar if not identical to those already occupied by the pre-existing corticofugal projections. This coupled scaffold system may be crucial in the development of area-specific thalamocortical connectivity. In culture, E15-16 cortical explants innervate thalamic explants but again there is no sign of positional preference. Therefore, we suspect that organized pathways in the extracellular matrix or simple mechanical factors may play important roles in the establishment of thalamocortical connectivity at early stages, when the forebrain is very immature.

416.12

INGROWTH OF THALAMOCORTICAL AXONS INTO EMBRYONIC RAT NEOCORTEX S. Catalano* and H.P. Killackey, Depts. of Anatomy and Neurobiology and of Psychobiology, University of California, Irvine, CA 92717

The growth of thalamocortical axons into rat neocortex was examined using the fixed brain DiI technique (Godement et al, *Development* 101:697, 1987). On the sixteenth day of gestation, E 16, thalamic axons can be traced into cortex and reach approximately halfway up the lateral-to-medial extent of the cerebral vesicle. At this age, a lamina of cells condensing underneath the cortical plate (presumably lamina 6b, Valverde et al, *J.Comp.Neurol.* 290:118, 1989) can begin to be distinguished. Thalamic axons run tangentially within the intermediate zone without branching. Many fibers course directly underneath the cortical plate and are thus in contact with lamina 6b. By E 18, lamina 6b is quite distinct, being separated from the cortical plate by a cell-sparse zone. Thalamic axons grow tangentially within lamina 6b as well as the intermediate zone below, and a dense layer of thalamic arbors can be seen growing radially within the cell-sparse zone, between the cortical plate and lamina 6b. By E 20 presumptive layer 6a has begun to differentiate and thalamic arbors continue to grow radially through it, but do not penetrate the cell-dense cortical plate. At no point are afferent fibers observed within the marginal zone. Thus thalamic axons are present within cortex from a very early age, and the growing arbors are in a position to contact target cells as these laminae differentiate from the cortical plate. (Supported by NSF grant BNS 87-19311).

416.13

CULTURED RAT RETINAL GANGLION CELL NEURITES DISPLAY AXONAL AND DENDRITIC MARKERS. Dana Leifer, Michael Marciello, Evan B. Dreyer, and Stuart A. Lipton. Department of Neurology, Children's Hospital, Massachusetts General Hospital, and Harvard Medical School, Boston, MA.

We have previously shown that neurite outgrowth by postnatal rat retinal ganglion cells is enhanced on substrates coated with monoclonal antibodies against the cell-surface glycoprotein Thy-1 (Leifer et al., *Science* 1984;224:303). These antibodies are specific in retina for the ganglion cells. We have also found that growth is increased if the cells are grown on an astrocyte monolayer or in the presence of acidic fibroblast growth factor, nicotinic antagonists or NMDA antagonists. We have now labeled retinal ganglion cells in culture with antisera (provided by Drs. K. Kosik and G. Lee) against the cytoskeletal proteins MAP-2 and tau, which are normally localized to dendrites and axons, respectively. Retinal cultures grown for one day on substrates coated with monoclonal antibodies against Thy-1 (supplied by Dr. C.J. Barnstable) were stained with these antisera. Double labeling with the anti-Thy-1 antibodies was used to identify the ganglion cells and their neurites. Immunoreactivity for both cytoskeletal proteins was found in essentially all ganglion cell neurites. This result indicates that after one day in culture, retinal ganglion cell neurites, and, in particular, those whose growth is enhanced by antibodies against Thy-1, have both axonal and dendritic features. The results also suggest that the study of these cultured neurons is relevant to conditions such as Alzheimer's disease in which MAP-2 and tau co-localize to abnormally proliferating neurites.

416.14

EFFECTS OF THYROXINE ON PROCESS OUTGROWTH FROM XENOPUS CNS EXPLANTS. D.L. Norris, M.S. Beattie, and J.C. Bresnahan. Depts. Anatomy and Surgery, and Neuroscience Program, Ohio State Univ., Columbus, OH 43210

Previous studies in our laboratory provide evidence for metamorphosis-dependent spinal cord regeneration in Xenopus tadpoles (Beattie et al., SN Abs. 13:972, 1987). We report here preliminary results of experiments to assess the role of thyroxine in axonal outgrowth from Xenopus CNS explants. Stage 50-54 brainstem - spinal cord explants, and juvenile spinal cord/DRG explants, survive and adhere to poly-L-lysine or Matrigel substrates when cultured in a modified L-15 medium (e.g., Grant & Tseng, Dev. Biol. 114:475, 1986). Retinal explants thrive under similar conditions. Tri-iodothyronine (T3), when added at a concentration of 10^{-8} M to stage 50 tadpole CNS explants severed at the brainstem - spinal cord junction, supported substantially greater process outgrowth and connection between the brainstem and spinal cord than did T4 added at similar concentrations. Dose-response studies and histological characterization of explant cultures are underway. (Supported by NS-10165 and OSU Surgery MRDF)

416.15

A POSSIBLE ROLE FOR RETINOIC ACID IN THE DEVELOPMENT OF SPINAL CORD INTERNEURONS. T. Shiga, V. P. Gaur*, K. Yamaguchi* and R. W. Oppenheim, Department of Neurobiology and Anatomy, Wake Forest University, Winston-Salem, NC 27103

We have been examining the development of interneurons in the chick spinal cord with the goal of elucidating the cues that guide axons to their targets. The earliest developing interneurons are divided into two groups; primitive longitudinal (PL) cells in the ventral region with rostrocaudal projection and circumferential (C) cells in the dorsal and lateral regions with ventrally projecting axons which join the ipsilateral or contralateral longitudinal pathway. Recent data indicates that retinoic acid is involved in various aspects of morphogenesis. We have examined the expression of cellular retinoic acid binding protein (CRABP) in the spinal cord between stages 12 and 44 (embryonic day (E) 2 and E18). Both PL-cells and C-cells expressed CRABP shortly after their final mitosis. With the onset of axonal growth, CRABP was observed both in cell bodies and axons, including growth cones. CRABP expression in cell bodies continued up to E18, whereas immunostaining of axons was greatly diminished. In vivo perturbation studies involving treatment with retinoic acid are in progress.

416.16

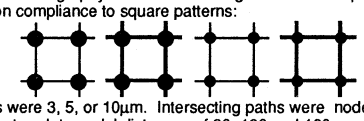
NEURITOGENESIS IN CENTRAL AND PERIPHERAL NERVOUS TISSUE COOLED BEFORE EXPLANTION. Nestor G. Carri and Ted Ebendal, Department of Developmental Biology, Box 587, Biomedical Center, S-751 23, Uppsala University, Uppsala, Sweden.

Neural transplantation techniques requires optimal tissue preservation. At present it is not known to what extent the time elapsed between dissection and the explantation to culture or implantation to a host affects the donor tissue. Neurite formation offers one possibility to study the integrity of the nervous tissue. We therefore studied neuritogenesis in embryonic neural explants using a short-term bioassay. After cooling dissected tissues to 8 °C in basal medium for different periods (24, 48, 72 h), the explants were cultured on hydrated collagen lattices. Tissues included neural retina and sympathetic ganglia from the chick embryo and dorsal root ganglia from an aborted human embryo. The explanted tissues received neurotrophic support during culture: Tectal extract was used for the retina and mouse βNGF for the ganglia. Neurites developed well and neuron survival was high after four days of culture using these conditions. The explants subjected even to the longest cold periods did not visibly deviate in their ability to form neurites. Our results demonstrate that nervous tissue can be preserved cold for a considerable period before being put into culture. This observation opens the possibility of future use of nervous tissue collected or manipulated several days before applied as grafts in brain surgery.
Supported by the Swedish Natural Science Research Council and TWAS,RG-BC 89-41.

416.17

COMPLIANCY OF HIPPOCAMPAL NEURONS GROWN ON PATTERNED MICROCIRCUITS. J.M. Corey[1], B.C. Wheeler[1], and G.J. Brewer[2]. [1]Neuroscience Program, University of Illinois, Urbana, IL 61801 and [2]Southern Illinois University School of Medicine, Springfield, IL 62794.

The study of synaptic specificity of CNS neurons would be facilitated by limiting their number and observing connections formed during directionally restricted growth of their axons and dendrites. Growth direction can be controlled by culturing neurons at low density on patterned substrates. Rat hippocampal neurons were dissociated and grown on these substrates at low density (6000 cells/cm^2) in defined medium. Substrate patterning was done by etching poly-lysine coated glass substrates with a UV laser through a quartz mask fabricated with electron beam lithography. We have investigated effects of path width and node size on compliance to square patterns:



Path widths were 3, 5, or 10μm. Intersecting paths were nodes of 5, 10 or 20μm diameter. Internodal distances of 80, 120 and 160μm were created. After 3 days of growth, the 80μm length patterns showed maximum adhesion (94%) to the 5μm path and 20μm node pattern. This was 50% better than the least compliant combination of 3μm paths and 5μm nodes. Somal migration to nodes from paths and from off-path areas was noted from observations over a four day period. These findings demonstrate effective neuron positioning on microcircuits by a process of selective adhesion and migration which will aid in later applications to localize neurons over substrate electrodes. Supported by Pearson Family Foundation and NIH BRSG funds.

416.18

NEUROBLASTOMA DIFFERENTIATION IS INFLUENCED BY ELECTRO/CHEMICAL MODIFICATION OF TISSUE CULTURE SUBSTRATES. V. Guénard, R.F. Valentini, S. Makohliso* and P. Aebischer. Section for Artificial Organs, Biomaterials and Cellular Technology, Brown University, Providence, RI 02912.

Numerous studies have demonstrated the importance of tissue culture (TC) substratum properties in determining neuronal attachment and morphology. Thus far, it has not been possible to determine whether the chemical and/or electrical characteristics of the substratum are responsible for morphological changes. The use of chemically and electrically modified polymeric growth substrates may provide insight into these mechanisms. Amine or oxygen rich polystyrene (P) TC dishes are produced by a plasma discharge process. The stable amine or oxygen groups on the P surface result in significant external positive (PP) or negative (NP) electrostatic fields, respectively. Polytetrafluoroethylene (T) can be made into positively (PT) or negatively (NT) charged electrets by injecting and trapping monopolar charges within the polymer bulk. The corona poling process used to fabricate T electrets does not induce chemical modifications on the T surface. Thus, PT and NT electrets generate significant external fields but have chemically identical T surfaces. Mouse neuroblastoma (Nb2a) cells were grown on P, PP and NP or T, PT and NT dishes for 24, 48, 72 and 96 hr. At all time points, Nb2a cells cultured on PP and NP showed increased neurite outgrowth and more flattened morphology as compared to standard P dishes. Nb2a cultured on PT showed better attachment and neurite outgrowth than NT, while unmodified T showed very little attachment. These results suggest that the electrostatic fields associated with different tissue culture substrates play an important role in regulating neuronal differentiation in vitro.

PROCESS OUTGROWTH, GROWTH CONES AND GUIDANCE MECHANISMS IX

417.1

MORPHOLOGICAL AND BEHAVIORAL DIFFERENCES BETWEEN GROWTH CONES FROM CULTURED SYMPATHETIC NEURONS AND ADRENAL CHROMAFFIN CELLS. K. L. Hoffman, C. A. Schulz and P. Claude†. Wisconsin Regional Primate Research Center and †Neuroscience Training Program, UW-Madison 53715.

Cultured adrenal chromaffin cells respond to nerve growth factor (NGF) by extending neurites and, after 14-21 days, transdifferentiating into sympathetic neurons. We enzymatically dissociated adrenal medullae or superior cervical ganglia from 8-day old rats, and plated cells onto collagen-coated glass coverslips set into 35 mm plastic culture dishes (Corning). The growth medium (DMEM;GIBCO) was supplemented with 0.6% glucose, 10% dextran-charcoal-stripped fetal calf serum (Hyclone), 100 μg/ml Gentamicin and 25 ng/ml 7S NGF (Collaborative Research). Using phase optics and time lapse video microscopy, we compared the morphology and motile behavior of growth cones from sympathetic neurons, from naive chromaffin cells and from chromaffin cells that had been exposed to NGF for times up to two weeks. In order to examine growth cones from chromaffin cells at different stages of neuronal differentiation, we replated cells after various times in NGF; when these cells are replated they immediately start to extend neurites tipped with growth cones. *Results:* Growth cones from mature sympathetic neurons do not adhere to the substratum over large areas; they are highly dynamic at the leading edge, with exuberant filopodial and lamellipodial activity. In contrast, after 3-10 days in NGF, chromaffin cells exhibit growth cones that are broad and flat, suggesting a uniformly high adherence to the substratum, and few, if any, filopodia. However, after 14 days of exposure to NGF, many of the replated cells extend growth cones that are indistinguishable from those of mature sympathetic neurons. This suggests that a developmental change in growth cone-substratum interaction takes place as these cells differentiate into neurons. *(Supported by NSF BNS-8616958 to P.C. and NIH RR00167 to the WRPRC.)*

417.2

DIFFERENCES IN Ca2+ HOMEOSTASIS IN GROWTH CONES AND SOMA OF AN IDENTIFIED NEURON: Na+-DEPENDENT AMPLIFICATION OF Ca2+ SIGNALS IN GROWTH CONES. J. R. Jensen, V. Rehder, and S. B. Kater. Dept. of Anatomy and Neurobiology, Colorado State Univ., Fort Collins, CO 80523.

The kinetics of the responses to perturbations of the intracellular calcium concentration ([Ca2+]i) differed greatly in neuronal growth cones (GCs) and cell bodies. The large GCs of cultured *Helisoma* buccal ganglia neuron 5 were utilized to compare Ca homeostasis in GCs to the soma of the same neuron. The fluorescent Ca2+ indicator Fura 2 (K+salt) was employed to assess [Ca2+]i. .

The Ca2+ ionophore, 4-bromo A23187, was used to increase Ca2+ influx and release from intracellular stores. The continuous presence of the ionophore triggered a biphasic response in the soma; a rapid transient increase was followed by a slowly increasing component. (Only the initial transient was still observed in Ca2+-free media, indicating that this component is due to release from stores.) In contrast, GCs exhibited a larger magnitude peak, intermediate in time to the two components of the cell body response. Moreover, this peak in GC [Ca2+]i was dependent on external Na+, while the cell body [Ca2+]i was only minimally affected by the presence of external Na+ at this dose of ionophore. Thus, when the Na+ content of the media was reduced from 45 to 5mM the sharp peak in GC [Ca2+]i was not observed; rather there was only a gradual rise to a plateau level similar to that eventually achieved in normal media. A similar Na+-dependent amplification in GCs was also observed in response to increases in [Ca2+]i evoked by the proton ionophore FCCP.

The results demonstrate that Ca2+ homeostasis is quite different in neuronal GCs and cell bodies. One factor contributing to this difference is a Na+-dependent system for amplification of increases in GC [Ca2+]i. The amplification that is observed could be produced by Ca2+ influx via the Na+-Ca2+ exchanger, as this system possesses positive feedback qualities that are consistent with our findings, although other possible mechanisms have not yet been ruled out.

417.3

THE EFFECT OF ELECTRICAL STIMULATION AND GROWTH-CONE-CALCIUM ON NEURITE OUTGROWTH IN RAT SYMPATHETIC NEURONS [1]T. K. Garyantes, [1]J. Pine, & [2]W.G. Regehr [1]Caltech, Pasadena CA 91125, [2]AT&T Bell Labs, Murray Hill NJ 07974.

Increasing growth-cone-calcium levels ($[Ca]_{gc}$) by electrical activity has been proposed as a mechanism to halt neurite outgrowth (Cohan etal., J. Neurosci., 7, 11, p.3588, 1987). We investigated the effect of increased electrical activity and $[Ca]_{gc}$ on the growth rate of cultured neonatal rat superior cervical ganglion neurons (SCGs). These neurons show low levels of spontaneous activity in control saline. We found that stimulation at 10 Hz for 1 hour, growth in high K^+ medium or high K^+/high Ca^{++} medium had little effect upon growth rates. Immediately following the onset of stimulation, $[Ca]_{gc}$ (measured using fura-2) transiently increased to greater than 500nM, but for all conditions tested sustained $[Ca]_{gc}$ remained below 250nM. These results sharply contrast those reported for cultured adult *Helisoma* neurons where electrical stimulation produced slightly larger $[Ca]_{gc}$ increases but stopped outgrowth. Intriguingly, for SCGs growth rates did not correlate with calcium levels in the range studied (50-250nM). These findings indicate that SCG outgrowth is unlikely to be greatly influenced by electrical activity at frequencies as high as 10Hz. Differences in calcium buffering systems, density or type of voltage-dependent calcium channels, or sensitivity of outgrowth to $[Ca]_{gc}$ between rat SCGs and *Helisoma* neurons may explain their different responses to electrical stimulation.

417.4

Filopodial Formation in Neuronal Growth Cones Examined With a Laser Trap. D.B. Wayne,S.C. Kuo*,N.L. Baumrind,A.L. Pearlman† and M.P. Sheetz* Depts of Cell Biology and Neurology†, Washington University Medical School, St. Louis, MO 63110

We have used the radiation pressure of a laser trap to manipulate antibody- or protein-conjugated (0.5μm) latex beads on the surface of embryonic murine cortical growth cones. With the laser trap, beads attached to the cell surface can pull novel membranous extensions (neopodia) which resemble filopodia in their general form. Release of neopodia from the laser trap resulted in 3 types of response: an instant elastic recoil; an immediate but slow recoil; or a rigid form which extended, moved, or actively retracted. The formation of a rigid structure suggests conversion to a true filopodium. The response observed depended primarily on the activity of the growth cone prior to neopod formation: motile growth cones produced rigid neopodia. We saw no evidence that cytoskeletal rigidity forms from the distal tip inward; instead it progressed from the proximal end outward. Thus, mechanical extension of membrane can induce formation of active filopodia, which presumably requires actin polymerization. This occurs with greater frequency on active rather than quiescent growth cones, suggesting that specialized structures or conditions exist in the active growth cone.

417.5

A CONDITIONING FACTOR RELEASED FROM GANGLIA PROMOTES NEURITE OUTGROWTH OF CULTURED APLYSIA NEURONS. M. Fejtl, C. Trautman* and D. O. Carpenter. Wadsworth Labs and School of Public Health, Albany, NY 12201.

We have investigated the effect of ganglion-derived conditioned media (CM) on Aplysia neurite outgrowth in culture using a variation of the protocol reported for Helisoma neurons (Wong, et al., J. Neurosci., 1:1008, 1981). Periesophageal ganglionic rings from six Aplysia were placed in 4 ml of L-15 media for 72 hrs to extract the conditioning factor (CF). Culture dishes with poly-L-lysin coated cover slips were exposed to the CM for 24 hrs (1 ml/dish) by passing the CM through a Gelman 0.2 μm filter and rinsed in L-15 media. Neurite outgrowth in control and CF dishes was examined after 24 hrs. Only neurons which possessed at least two neurites with a length twice the diameter of the cell body were considered. The total length of the largest neurite of each cell was measured and compared within the two groups. Every cell cultured on the CF showed enhanced neurite outgrowth compared to the control group. For quantification neurite length measurements were made on 15 neurons of each group. Neurite length (Mean ± S.D.) of the control group was 261 μm ± 89 and 555 μm ± 107 for the CF group. We concluded that a CF released from Aplysia ganglia is a growth factor distinct from any found in the hemolymph.

417.6

NGF-INDUCED PC12 CELL GROWTH CONE MOTILITY AND ITS DIFFERENTIAL INHIBITION BY PURINE ANALOGUES. K. Phelan, C. Volonté and L. A. Greene. Department of Pathology and Center for Neurobiology & Behavior, College of Physicians & Surgeons, Columbia University, New York, NY 10032.

The growth cones of living PC12 cells were examined using Video Enhanced Contrast-Differential Interference Contrast (VEC-DIC) microscopy. During observation, cells were contained in a sealed chamber and could be continuously superfused with medium containing various additives. Thus, this system is useful for observation of and pharamacological intervention in the mechanism of action of NGF on the growth cone.

In the presence of NGF, PC12 cells extended neurites which were tipped by active, motile growth cones. Deprived of NGF overnight, PC12 growth cones became blunt and ceased lamellipodial extension. Upon readdition of NGF, growth cones reactivated after one minute's exposure to the growth factor. Reactivation included protrusion of many microspikes from the body of the growth cone and extension of broad lamellipodia from the leading edge.

The purine analogues 2-aminopurine (2-AP) and 6-thioguanine (6-TG) have been shown to block some but not all of NGF's actions on PC12 cells via differential inhibition of protein kinases (Volonté, C. et al., J. Cell Biol., 109:2395, 1989). Short-term (10 minute) pretreatment of PC12 cells with 2-AP blocked NGF-induced growth cone reactivation in a dose-dependent manner. The concentrations tested were sufficient to block other PC12 responses to NGF, including neurite regeneration. In contrast, short term (10 minute) treatment with 6-TG did not inhibit growth cone reactivation. Long-term (1 day) treatment of PC12 cells with 6-TG resulted in neuritic retraction, compatible with 6-TG's ability to block NGF-dependent neurite regeneration.

This blockade by one of a family of compounds known to inhibit protein kinases in these cells implies that phosphorylation is involved in the regulation of growth cone motility. We can exclude the NGF-regulated serine protein kinase (PKN) from this process since it is relatively specifically inhibited by 6-TG. 2-AP, a more general inhibitor, exerts its effects in the growth cone through blockade of kinases distinct from PKN.

417.7

INDUCTION OF NEURITE OUTGROWTH BY BRIEF EXPOSURE TO 12-O-TETRADECANOYL PHORBOL 13-ACETATE (TPA). S. Mehta*, L. Hsu, A.Y. Jeng* and K.Y. Chen*. Chem. Dept.,Rutgers Univ.,Piscataway,NJ 08854; Biol. Dept.,Seton Hall Univ.,S.Orange,NJ 07079; Research Dept.,Pharma.Div.,CIBA-GEIGY Corp., Summit,NJ 07901.

The time-course of neurite-promoting effects of TPA on sensory ganglia of White Leghorn chick embryos was investigated. Brief exposure of ganglia to 20-200 ng TPA/ml growth media for 30 minutes was sufficient to elicit neurite outgrowth and fasciculation. These responses were only observed in ganglionic explants that had attached to a collagen substrate prior to TPA treatment. Incubation of unattached ganglia with TPA was ineffective. This lack of response to TPA from unattached ganglia was not related to uptake of the phorbol ester. ^{35}S-methionine pulse labelling studies revealed that the synthesis of two proteins with molecular weights of 43 and 63 kD was enhanced by TPA treatment. These results suggest that activation of protein-kinase C may be involved in the initial phases of neurite differentiation.
(Supported by NS 21262)

417.8

KCl DEPOLARIZATION TRANSIENTLY ALTERS GROWTH CONE MOVEMENTS OF CULTURED *HELISOMA* NEURONS . C.S. Cohan and M.-H. Xia*. Dept. of Anatomical Sciences, SUNY Buffalo, Buffalo, New York 14214.

Previous experiments have demonstrated that signals such as electrical activity and neurotransmitters alter neurite elongation and growth cone movements of cultured *Helisoma* neurons. These signals cause their effects by depolarizing neurons, thereby opening voltage-sensitive Ca^{2+} channels, and increasing intracellular Ca^{2+} in growth cones. The present experiments studied the effects of long term depolarization induced by KCl application on growth cone movements.

Identified neurons B4, B5, and B19 were isolated from the buccal ganglia of *Helisoma* and plated into culture dishes containing a neurite promoting conditioned medium. Twelve hours after plating, time-lapse photographs at 15 min intervals were used to quantify rates of growth cone advance over a 135 min observation period. Rates of growth cone advance were assessed for the first 45 min (baseline) period and then over two successive experimental periods (45-90 and 90-135 min). Small doses of KCl (final concentrations 5, 10, 25, and 50 mM compared to 1.7 mM of culture medium) were added to the dish at the end of the baseline period.

The addition of KCl to culture medium caused a dose-dependent decrease in the rate of neurite elongation of B4 during the 45-90 min period. Inhibition of elongation steadily increased over the range 5 to 50 mM KCl with the two highest concentrations producing significant neurite retraction. In contrast, during the 90-135 min period, elongation rates spontaneously recovered toward the baseline rate. Rates during this period were slower than the baseline rates except for 10 mM KCl which resulted in an enhanced rate. Similar effects were observed for B19 and B5 although each neuron had different thresholds and quantitative relationships between KCl concentration and elongation rate and only B4 exhibited the spontaneous enhancement of elongation. The addition of Co^{2+} blocked the KCl induced suppression of elongation. These data indicate that growth cones spontaneously recover their motile properties while in the sustained presence of a depolarizing stimulus.

(Supported by NIH grant NS25789)

417.9

CYTOSKELETAL REORGANIZATION IN RESPONSE TO CONDITIONING FACTORS IN REGENERATING *HELISOMA* NEURONS. D.L. Kania and C.S. Cohan Dept. of Anatomical Sciences, SUNY at Buffalo, Buffalo, N.Y. 14214

Little work has been done to describe the initial events at the cut end of the axon during initiation of regeneration. This study examines the cytoskeletal changes at the end of the axon signaled by conditioning factors which result in the initiation of neuronal growth.

Neurons B19, B4, and B5 were removed from buccal ganglia of the mollusc, *Helisoma trivolvis*, with an attached piece of axon. When plated onto a polylysine substrate in defined medium, a growth cone formed at the end of the axon stump. This growth cone did not advance across the substrate but exhibited lamellipodial and filopodial movements. Fluorescent labeling of f-actin and tubulin revealed a characteristic pattern of cytoskeletal elements. Thus, these growth cones were indistinguishable from growth cones formed in the presence of conditioned medium.

The typical response to axotomy is the extension of many new neurites from a single axon stump. After the addition of conditioning factor, many new neurites extended from the periphery of the growth cone formed in defined medium. The same response was seen for isolated growth cones suggesting a local effect at the growth cone. Cytoskeletal staining revealed a breakdown in the peripheral actin network in the growth cone and accumulation of f-actin in areas where new neurites formed. Microtubules extended to the edge of the growth cone into an area normally devoid of microtubules.

These data indicate that growth cone formation is independent of the initiation of outgrowth. Outgrowth is initiated by conditioned medium which signals changes necessary for neurite extension.

(Supported by NIH grant #NS25789)

417.10

CALCIUM LEVELS DO NOT CHANGE DURING CONTACT MEDIATED GROWTH CONE COLLAPSE. J.K. Ivins, J.A. Raper and R.N.Pittman. Depts. of Pharmacology and Anatomy, U. of Pennsylvania School of Medicine, Philadelphia, PA 19104.

The growth cones of chick DRG neurons undergo a collapse of structure when they contact the neurites of chick retinal ganglion cells in culture. We have used the calcium indicator dye fura-2 and low light level digital imaging fluorescence microscopy to ask whether this growth cone collapse is mediated by increases in growth cone calcium. We find that calcium levels in DRG growth cones are very stable during contact and collapse. The possibility remained that small increases in calcium levels occur but are below the level of detection for our optical system or that transients of calcium occur at rates greater than our sampling rates. However, the calcium ionophore ionomycin (1 μM) raised growth cone calcium levels 2-3 fold, but had no effect on growth cone morphology, growth cone motility, or rates of growth cone advance. Furthermore, depolarization of cultures with 15 mM KCl caused 2-3 fold increases in growth cone calcium levels. These increases were transient in nature, typically lasting 60-90 seconds, but did not affect growth cone morphology, growth cone motility, or rates of growth cone advance. Growth cone calcium levels also remain stable during exposure to a crude growth cone collapsing activity derived from E10 chick brain membranes. Therefore, changes in growth cone calcium levels do not appear to account for the morphological features of contact-mediated growth cone collapse.
(Supported by NIH 22663 and the McKnight Foundation).

418.1

IDENTIFICAITON WITH A MONOCLONAL ANTIBODY OF A TEMPORAL RETINAL AXON PROTEIN IN DEVELOPING CHICK. S.C. McLoon. Univ. of Minnesota, Minneapolis, MN 55455.

In the process of generating monoclonal antibodies to chick retinal axons, one antibody was found that labels by immunohistochemistry most, if not all, axons in temporal retina. The majority of axons in nasal retina were not recognized by this antibody. A similar dicotomy in the staining of neurites from explants of nasal and temporal retina was seen within the first few days in culture. The neurites of living temporal retinal explants stained without permeablizing the membranes suggesting that the antigen is a cell surface molecule. Immunoblots of embryonic retinal tissue revealed a tight band with an approximate mass of 135kD. The antibody did not bind to the antigen after treatment with trypsin suggesting that it is a protein. The division between the temporal and nasal retina as revealed by this antibody corresponded to the optic fissure. The immunohistochemistry suggested a sharp division between the two sides of the retina in terms of the concentration of this antigen, rather than a continual gradient of the antigen from one side to the other. A sensitive competition based ELISA was developed to quantify the amount of this antigen on axons in different regions of the retina. This confirmed the existence of a step gradient of this antigen. Our working name for this molecule is TRAP for temporal retinal axon protein. TRAP is the first molecule identified in an asymmetric distribution in the nasal-temporal axis of retina. It remains to be determined what role, if any, TRAP plays in development of the pattern of connections between the eye and the brain.

418.2

P-BRAVO, A NOVEL MEMBER OF THE IMMUNOGLOBULIN SUPERFAMILY, IS FOUND IN A RESTRICTED PATTERN ON OPTIC FIBERS OF THE DEVELEOPING NERVOUS SYSTEM. J. F. Kayyem, E. J. de la Rosa*, J. M. Roman*, U. Schwarz* and W. J. Dreyer*. California Institute of Technology, Pasadena, CA 91125.

Cell-surface molecules play an essential role in guiding axons to their targets. We have developed a method to generate monoclonal antibodies (mAbs) which recognize cell-surface molecules of defined molecular weight that are expressed during the development of the chicken nervous system. Fourteen distinct spatial and temporal patterns of tissue reactivity were detected with mAbs generated against cell-surface molecules of approximately 135 kD. The antigen distribution recognized by one of these mAbs, pattern Bravo (P-Bravo), results from the topologically restricted labeling of optic fibers. In the retina, optic fibers are labeled by P-Bravo mAbs. However, these same fibers are not labeled by P-Bravo in the tectum. P-Bravo antigen, therefore, appears to be restricted to the proximal, and absent from the distal, axon.

Protein analyses show that P-Bravo antigen is a member of the immunoglobulin superfamily, contains fibronectin type III related sequences, and carries the HNK-1 epitope, thought to be involved in cell adhesion. P-Bravo is closely related to the neural cell surface molecule L1, but is not G4, thought to be the chicken equivalent of L1. The homology of P-Bravo to known adhesion molecules, as well as the restricted pattern of antigen distribution, suggests P-Bravo is involved in axon guidance.

418.3

STRUCTURAL AND FUNCTIONAL ANALYSIS OF L1 VIA PROTEOLYTIC CLEAVAGES. A. Abosch and C. F. Lagenaur. Dept. of Neurobiology, Anatomy, and Cell Science, Univ. of Pittsburgh, Sch. of Med., Pittsburgh, PA 15261.

In order to determine the location of the L1 active site, a series of proteolytic digests were performed on affinity-purified L1. The resulting peptides were analysed by SDS-PAGE and Western blot using monoclonal antibodies specific for the 135 and 85 kD fragments of the molecule. The cleavage products were then spotted on nitrocellulose-coated dishes, and dissociated mouse cerebellar cells added in order to assess neurite-outgrowth promoting activity (Lagenaur and Lemmon, PNAS 84:7753-57, 1987). Elastase digestion of L1 resulted in retention of the 135 kD fragment, while eliminating the 85 kD fragment along with the activity of the molecule. Papain digestion eliminated all trace of the 135 kD fragment on Western, but portions of the 85 kD fragment, as well as the activity of the molecule, were retained. These data provide evidence for the importance of the membrane-spanning 85 kD fragment in the function of L1. Supported by NIH NS25543.

418.4

CELL ADHESION MOLECULES 8D9 AND NCAM MOVE INDEPENDENTLY IN THE PLANE OF AXON MEMBRANES. J. DRAZBA[1] & V. LEMMON, Laboratory of Neurobiology[1], NINDS, NIH, & Dept. of Neuroscience, Case Western Reserve University, Cleveland, OH, 44106

Neuronal cell adhesion molecules may be functionally cooperative (Kadmon et al., J. Cell Biol. 110:193-208) and may therefore be physically linked. To test this idea, we investigated the localization of 8D9 (a member of the L1/NILE /NgCAM/G4 family of cell adhesion molecules) and NCAM, two integral membrane proteins, on the surface of chick retinal ganglion cell axons. Polyclonal antibodies to either 8D9 or NCAM were added to live cultures of E7 chick axons (Drazba & Lemmon, Dev. Biol. 138:82-93) which were subsequently fixed and incubated with monoclonal antibody (Mab) against the other adhesion molecule. The cultures were then treated with separate secondary antibodies in order to visualize the distributions of both cell adhesion molecules. Examination of the axons and growth cones with a Bio-Rad MRC 600 Laser Scanning Confocal Microscope revealed that the 8D9 or NCAM, exposed to the polyclonal in the living cell, had patched, while the other cell adhesion molecule, labelled post-fixation with the Mab, was distributed evenly over the surface of the same axon. Control cultures fixed before being exposed to both primary antibodies exhibited uniform distributions of both antibodies. These results suggest that 8D9 and NCAM are free to move independently of one another in the plane of the membrane and so are not necessarily physically linked.

418.5

MOUSE cDNA CLONE FOR THE NEURONAL CELL-SURFACE ADHESION MOLECULE P84. R. Perez, C. Lagenaur and R.M. Lewis. Dept. NACS, Univ. Pittsburgh, Pittsburgh, PA 15261.

P84 is a neuronal cell surface adhesion molecule which effectively promotes neurite outgrowth. Antibodies to P84 label only the floor plate in early embryogenesis in the mouse. Label is found throughout the adult brain. To determine whether this label is due to P84 in cell bodies or axons, we attempted to clone the cDNA for P84 for use in *in situ* hybridization. A mouse brain cDNA expression library in λgt11 was screened with antibodies to P84. Of the nine clones obtained, seven cross-hybridized. The fusion product of this most common clone bound antibodies to P84 on western blots. All clones were less than full length. None of the clones had sequences homologous to recorded sequences. A 23 amino acid sequence (which was not coded for by any of the nine clones, and which was not homologous to any recorded sequence) was obtained from purified mouse P84. A synthetic oligonucleotide derived from this sequence was used to screen the library. Four clones were confirmed by progressively more stringent hybridizations with the oligonucleotide. Sequences for these clones are being determined. The clones are being used to determine the size of the P84 mRNA by northern blots. The number of genes coding for P84 is being determined initially by genomic southern blots. Once the entire cDNA for P84 is obtained, it will be utilized to map active regions of the molecule.

418.6

CELL-TYPE SPECIFIC PERIPHERAL AND INTEGRAL MEMBRANE PROTEINS AND THEIR COMPLEMENTARY MANNOSE-BINDING PROTEIN IN THE LEECH CNS. R.N. Cole and B. Zipser. Department of Physiology, Michigan State University, E. Lansing. MI 48824.

In leech neuronal development, sensory afferents entering the synaptic areas of the CNS defasciculate as they disperse to their connections. This defasciculation is mediated by the interaction between a mannose-containing epitope on the sensory afferent glycoprotein and a putative mannose-binding protein (Zipser and Cole, Neurosci. Abst. 1989). Sensory afferents express this mannose-containing epitope on 3 different proteins (130, 103 and 95 kD). We determined the membrane topology of the 3 sensory proteins by phase separation using Triton X-114. The majority of the 130 kD protein is found in the aqueous phase, behaving as a peripheral membrane protein, and is expressed early during the time of target recognition (McGlade et al., J. comp. Neurol., in press). In contrast, the 103 and 95 kD proteins are found in the detergent phase, behaving as integral membrane proteins, and are expressed after synapses are established. To identify the putative mannose-binding protein that interacts with the sensory afferent protein, we fractionated leech CNS by gel filtration and affinity chromatography. Gel filtration studies indicate that the sensory protein from CNS homogenized in the presence of mannose, but not galactose, elutes, as expected, in lower molecular weight fractions. This is preliminary evidence that the 130 kD peripheral sensory protein binds to a mannose-binding protein on leech membranes.

418.7

DISTRIBUTION OF TENASCIN IN IMMATURE AND MATURE HUMAN BRAIN. R.D. McComb and K.A. Miller*. Department of Pathology and Microbiology, University of Nebraska Medical Center, Omaha, NE 68198.

Tenascin (TN) is an extracellular matrix glycoprotein involved in cell adhesion and migration. In this study the distribution of TN was examined immunohistochemically in 36 human brains (cerebrum and cerebellum) ranging from 17 weeks gestational age (GA) to 92 years of age. At 17-26 weeks GA, the intermediate zone of the cerebrum exhibited greater reactivity than the cortical plate. Beyond 30 weeks GA there was diffuse staining of both cortex and white matter which persisted into the first postnatal year. At older ages the molecular layer (ML) and white matter maintained strong reactivity, but the cortex showed only patchy perivascular staining. In immature cerebella, intense reactivity was observed in the ML and internal granule layer (IGL). Reactivity in the IGL decreased during the first postnatal year and was not detected at older ages. Reactivity in the ML persisted until the second decade, but was weak or absent at older ages. The dentate nucleus was reactive at all ages, often showing an intense perineuronal pattern suggestive of membrane localization. The temporospatial distribution of TN is compatible with its proposed role in cellular migration in the developing brain. Its persistent expression in adult brain and in reactive astrocytes following brain injury indicates additional functions for this glycoprotein.

418.8

FIBRONECTIN BINDING TO BRAIN SYNAPTOSOMAL MEMBRANE MAY INVOLVE NOVEL MATRIX RECPTORS. B.A.Bahr, A.Sheppard, P.W.Vanderklish*, B.Bakus*, D.K.Capaldi*, M.Kessler, L.T.Ha*, M.T.Tin* & G.Lynch. CNLM, University of California, Irvine, CA 92717 U.S.A.

Synapses presumably share properties with other types of cell-cell adhesion contacts. Integrins and other matrix receptors act as transmembrane links between the extracellular matrix and the cytoskeleton, forming focal contacts with fibronectin (Fn), vitronectin (Vn), laminin, and proteoglycans. [^{125}I]-Fn binds to rat brain synaptosomal membrane (SPM) with a specific activity of 5-10 pmol/mg protein and this was blocked by RGDS (1 mM), a compound known to bind integrin receptor sites. This result predicted the existence of synaptic integrins. Polyclonal (pAb) and monoclonal (mAb) antibodies toward human Fn-receptor (FnR) or VnR recognized previously described integrin subunits of 110, 175 (S175), and 225 (S225) kDa in brain homogenate, but these polypeptides were almost completely absent from SPMs. However, pAb against a fibroblast integrin (Schwartz et al., 1989) and mAb against an integrin epitope near the receptor site (Telios Pharm., Inc.) labeled synaptosomal peptide bands of 40 (F40) and 70 kDa. These antigens were $\geq$ 20-fold enriched in SPMs compared to brain homogenates, and could not be detected in various non-neuronal tissues. It remains to be determined if either species is the RGDS-sensitive fibronectin binding site in SPMs.

Calcium activated proteases (calpains) are concentrated at adhesion sites (Bennett et al., 1984) and it has been established that certain proteins thought to link integrins to the cytoskeleton are substrates for these enzymes. Preliminary studies indicate that calpain I degrades integrin subunits found in brain homogenates and also reduces the concentration of F40 in SPMs. These interactions might provide a route through which increases in intracellular calcium could reorganize neuronal connections.

418.9

HNK-1 ANTIBODY RECOGNIZES NEUROMUSCULAR JUNCTIONS. C.P. Ko, P.W. Wong* and L.A. Holcomb*. Dept. of Biol. Sci., Univ. of So. Calif., Los Angeles, CA 90089.

The monoclonal antibody HNK-1 recognizes a carbohydrate epitope and has been used to label migrating neural crest cells. We report here that HNK-1 also recognizes neuromuscular junctions (NMJs) in the frog and the chick. Double-labeling of skeletal muscles in whole mount or cryosection with fluorescent alpha-bungarotoxin, nerve terminal dye or Karnovsky's cholinesterase stain verified HNK-1 binding at the NMJ. HNK-1 staining outlines nerve terminals and acetylcholine receptors. The labeling appears similar to that of fluorescent peanut agglutinin (PNA), previously shown to recognize the extracellular matrix (ECM) at frog NMJ and myotendinous junctions. Unlike PNA, HNK-1 does not recognize myotendinous junctions but does stain small, possibly unmyelinated nerve fibers seen near NMJs and blood vessels. To localize HNK-1 binding sites, we denervated, froze and removed all but a thin bridge of muscle containing NMJs. HNK-1 still stained the synaptic site after nerve and muscle degeneration. Electron microscopy of muscle treated with HRP labeled HNK-1 showed reaction products in the ECM of the NMJ. Results suggest that the carbohydrate epitope recognized by HNK-1 exists in the synaptic ECM. Since this epitope is common to several adhesion molecules, HNK-1 may provide a probe to study the function of carbohydrates in synaptic differentiation and maintenance.

418.10

EXPRESSION OF THE SYNAPTIC CLEFT PROTEIN S-LAMININ IN CELL LINES. T.L. Green[1], D.D. Hunter[1], W. Chan*[1], J.P. Merlie[2], and J.R. Sanes[1] Depts. of Anatomy and Neurobiology[1], and Pharmacology[2], Washington Univ. Med. Sch., St. Louis, MO 63110.

S-laminin is a laminin-like glycoprotein that is concentrated in the basal lamina at the neuromuscular junction (Hunter et al., Nature 338:229, 1989). It bears a site to which motoneurons selectively adhere (Hunter et al., Cell 59:905, 1989), suggesting that it may be recognized by axons at synapses. However, tests of this hypothesis have been limited by lack of a source of native s-laminin; the functional assays used bacterially produced protein. We have therefore taken two approaches to identify and characterize s-laminin produced by cell lines. First, we screened a variety of rat cell lines by immunoblotting with monoclonal antibodies (mabs) to s-laminin. Several skeletal and smooth muscle cell lines (L6,L8,A10,RMO) which produce laminin also produce and secrete s-laminin. Second, we constructed an expression vector containing the entire s-laminin cDNA, and isolated stable transfectants in C2 (mouse muscle) and QT6 (quail fibroblast) cells. By immunofluorescence using rat-specific mabs, both cell lines accumulate s-laminin intracellularly. C2 cells secrete the recombinant protein and incorporate it into extracellular matrix after they fuse to form myotubes. We then immunoprecipitated s-laminin with subunit-specific antibodies to analyze the oligomeric structure into which it is assembled. Laminin is composed of homologous A, B1, and B2 subunits; s-laminin is most closely related to the B1 subunit. Antibodies to s-laminin coprecipitate laminin B2 and an A-like subunit, whereas antibodies to B2 precipitate B1, B2, s-laminin, and an A-like chain. These results support immunostaining data (Sanes et al., submitted) suggesting that s-laminin and B1 can substitute for each other in a trimer with A and B2. We are now purifying s-laminin to test this model and to study interactions of neurons with the complex. (Supported by NIH and Monsanto.)

418.11

LAMININ AND S-LAMININ ARE PRODUCED AND RELEASED BY ASTROCYTES, SCHWANN CELLS AND SCHWANNOMAS. A.Chiu, A.Espinosa*, R.Cole*, S.Loera & J.de Vellis*, Beckman Res.Inst.of City of Hope,Duarte,CA & *UCLA, Depts.Anat. & Cell Biol.L.A.,CA.

Laminin, a potent promotor of neurite outgrowth, is present in all extracellular matrices (ECMs). In contrast, s-laminin, a homolog of laminin, is highly localized at the neuromuscular synaptic cleft. While the distribution of these two ECM components have been well documented in situ, the sources of these molecules is unclear. We report that astrocytes cultured in serum-free medium maintain an organized ECM that only bears laminin immunoreactivity; s-laminin appears to be sequestered intracellularly. However, both molecules are found in Western blots of the astrocyte conditioned medium. Thus, astrocytes produce and release laminin and s-laminin, but only incorporate the former into an ECM. In contrast, neither molecule is present in cultures of oligodendrocytes. Although no established matrix is seen in cultures of Schwann cells or Schwannomas, laminin and s-laminin immunoreactivity are present within cells and in the conditioned media. These results indicate that certain populations of non-neuronal support cells and cell lines can produce and release both synaptic and extrasynaptic components of the ECM. Assembly of these different molecules into an organized ECM may require additional factors or interaction with neurons.

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418.12

LOCALIZATION OF A PLASMINOGEN ACTIVATOR AND ITS INHIBITOR AT ADHESION SITES OF CULTURED RAT MYOTUBES. G.M.Dmytrenko and M.B.Clark. Depts. of Neurology and Anatomy, Univ. of Maryland Sch. of Medicine, Baltimore, 21201

Cultured neonatal rat myotubes cluster AChRs, develop cytoskelton and membrane specializations, and organize extracellular matrix molecules where they adhere to the substrate. Since the protease plasmin is known to modify extracellular matrix components we asked whether controlling factors, such as plasminogen activators (PA's) or their inhibitors (PAI's) were positioned to influence the modification of the extracellular matrix at the myotube - substrate adhesion sites?

We used sequential detergent extraction and immunofluorescence microscopy to identify a urokinase - like PA (uPA) as well as a plasminogen activator inhibitor (PAI-1) at myotube - substrate adhesion sites. Adhesion sites isolated with saponin demonstrated uPA immunoreactivity in a linear pattern coincident with AChR-rich domains. PAI-1 immunoreactivity was present in surrounding culture substrate but apparently absent from the adhesion sites. When the isolated myotube - substrate adhesion sites were further treated with Triton X-100 to remove remaining cell membrane, uPA was detected at the adhesion sites, while PAI-1 was seen in the surrounding culture substrate and appeared reduced at the adhesion sites. Thus, PAI-1 is generally distributed in the extracellular substrate to prevent widespread proteolysis, while UK is focally positioned to affect changes in the extracellular composition of myotube - substrate adhesion sites.

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418.13

ASPECTS OF CALPAIN BIOCHEMISTRY AND NEURONAL MEMBRANE PLASTICITY. G.Lynch, J.Wu, B.A.Bahr & A.M.Sheppard. CNLM, University of California, Irvine CA 92717 U.S.A.

The calcium-dependent protease calpain is thought to play a role in the elaboration of structural plasticity in both erythrocytes (Siman et al., 1987) and neurons (Lynch and Baudry, 1984). We have investigated aspects of its biochemistry in crude and synaptic plasma membranes prepared from rat telencephalic tissue. Western blot analysis, utilising a polyclonal antibody generated against purified calpain I isolated from rat erythrocyte cytosol, revealed that the expression of an apparently activated form of calpain is developmentally regulated at the neuronal membrane surface during a period of considerable structural plasticity. Similar analysis demonstrated that a principle substrate, namely spectrin, was available at the submembraneous surface and susceptible to the proteolytic action of calpain throughout the postnatal developmental period studied. The action of calpain was found to cause a loss in the expression of a restricted number of membrane associated components. Nearly all of these were relatively large molecular weight Concanavalin A-binding glycoproteins and included the cell adhesion molecule N-CAM. The present findings are consistent with and support the postulated role of calpain in facilitating membrane plasticity.

418.14

DISTRIBUTION OF BRAIN SPECTRIN 240/235 AND 240/235E IN NEURONS IN HUMAN OCCIPITAL CORTEX. K.S. Pollan*, D.J. Woodward and M-C. de Lacoste. Dept. Cell Biology and Neuroscience, U.T. Southwestern Med. Sch., Dallas, TX 75235

This study was undertaken to determine if two isoforms (240/235, 240/235E) of brain spectrin, a membrane-associated protein implicated in neuronal plasticity and degeneration, are differentially distributed within neuronal compartments in human visual cortex. It has been proposed that the two distinct spectrin subtypes are differentially localized within dendrites, cell bodies and axons and, hence, that they may have different structural/physiological functions (e.g., Riederer et al., 1986; Goodman & Zagon, 1989).

Formalin-fixed blocks of tissue from human occipital cortex were sectioned at 50 µm in the coronal plane on a freezing microtome. Spectrin-containing neurons were immunolabelled using the avidin-biotin immunoperoxidase method and antisera to spectrin 240/235 and 240/235E (Chemicon, Inc.). Sections were then counterstained with a light Nissl. Human cerebellum served as a positive control.

In the human visual cortex, spectrin 240/235 immunoreactivity was prominent in axons. In addition, however, a small and scattered population of pyramidal and stellate-type neurons appeared to contain spectrin 240/235 within their soma as well as dendrites and axons. Spectrin 240/235E immunoreactivity was confined to the soma and dendrites of a small and scattered population of neurons. No axonal labelling was detected using this isoform. In brief, there appears to be some segregation of the two spectrin subtypes in that only the 240/235 isoform is localized in neuronal axons. However, neuronal soma and dendrites in human visual cortex contain both brain spectrin 240/235 and 240/235E. *Supported by HD21-711-01 (MCL) and Biological Humanics Foundation.*

418.15

MICROFIBRILLAR ADHESION COMPLEXES OF DEVELOPING NEURITES: LINKAGE TO THE CYTOSKELETON. S.A. Enam and W.L. Klein. Northwestern Univ. Inst. for Neuroscience, Evanston, IL 60208

The cytoarchitecture of developing nerve cell surfaces contains microfibrillar specializations that constitute adhesion complexes (PNAS 52:5206). These somewhat irregular microfibril (8-13 nm wide, varying lengths) can form "trans-junctional adhesion complexes (TACs)" at nascent junctions as well as connect the cell surface to the substratum. For avian retina cells (Dev.Brain Res. 51:205), TACs and other surface microfibrils have been found to be multimeric and include components of adheron such as heparan sulfate proteoglycan for e.g. The current study extends observations of cell surface adhesive microfibril to cultures of rat fetal brain cells and several cell lines including human. Of particular interest, TACs and other adhesive microfibrils have been found resistant to mild detergent treatments that extract membranes and cytosol. Whole mount EM of extracted cells showed that the microfibril were linked to the cytoskeleton, directly supporting the hypothesis that adhesive molecules establish trans-membrane linkage with intracellular elements. Such complexes conceivably could have signal transduction capability. Molecular analysis showed that, after extraction, the microfibril still were labeled by antibodies to adheron. Studies also were done showing that some adhesive microfibrils include amyloid precursor protein (APP), a molecule closely associated with the pathogenesis of Alzheimers disease.

418.16

MONOCLONAL ANTIBODY KHRI-5 TO HAIR CELL STEREOCILIA IMMUNOSTAINS A CYTOSKELETAL STRUCTURE IN CULTURED FIBROBLASTS. M.Ptok, J.W.Horn*, T.S.Nair*, T.E.Carey*, R.A.Altschuler. Kresge Hearing Research Institute, Univ. of Michigan, Ann Arbor, MI 48109

A murine monoclonal antibody developed after immunization with chick basilar papilla, guinea pig outer hair cells and frog lateral line specifically labels stereocilia in the inner ear. In the present study this antibody was applied to cultured fibroblasts where it labeled a fibrous network in the cytoplasm. Double labeling with phalloidin (marking filamentous actin) showed that KHRI-5 labeling was distinct from but associated with f-actin. Immunoelectron microscopy was then used to study ultrastructural localization. Permeabilized fibroblasts were incubated with KHRI-5 antibody and antigenic sites were visualized by 5 nm immunogold particles. Specific labeling was distributed in clusters in close proximity to 200 - 300 nm fibers. Since both hair cell stereocilia and fibroblasts express the epitope recognized by KHRI-5, they may have this cytoskeletal structure in common. Its characterization should be facilitated by the use of cultured cells.

(supported by NIH grant NS 05785 and Deutsche Forschungsgemeinschaft - Postdoctoral Fellowship for M.P.)

418.17

IN VIVO AND IN VITRO EXPRESSION OF CD4 IN FETAL HUMAN BRAIN. S. Torelli*, M.G.Ennas*, V.Sogos*, E.Pilia* and F.Gremo. Dept.Cytomorph.Med.Sch., 09124 Cagliari (Italy).

CD4 has recently attracted much interest since it is the receptor for Acquired Immunodeficency Syndrome (AIDS) virus (HIV). It is known that HIV can cross the placenta and infect the fetus. In order to investigate the possibility of a direct infection of fetal brain, we performed immunohistochemical localization of CD4 both at light and electron microscope in cultures of fetal brain from 8 to 20 weeks of gestation. Results showed that CD4 was present in both cytoplasm and cell membrane of neurons in the first 4 weeks in vitro. Later, CD4 was localized also in the cytoplasm of some, not all astrocytes. Immunoprecipitation studies showed the presence of a protein of molecular weight slightly lower than normal (45 Kd versus 60 Kd). These results were confirmed by molecular biology studies, which showed the presence of a transcript corresponding to a truncate form of the protein (lacking of the gp120-binding site) in early stages (from 12 to 18 weeks of gestation), whereas the complete protein was found in older fetal brains.

419.1

PROTEIN KINASE C MAY CONTRIBUTE TO THE INCREASE IN SPONTANEOUS RELEASE EVOKED BY 5-HT AT CULTURED *APLYSIA* SENSORY-MOTOR SYNAPSES. O. Braha, N. Dale*, M. Klein and E. R. Kandel. HHMI, Columbia, NY, NY 10032.

Presynaptic facilitation of the synapses between the siphon sensory and the gill motor neurons evoked by 5-HT involves at least two mechanisms: a first process that is a cAMP-dependent broadening of the presynaptic actin potential and a second process that may involve direct modulation of the transmitter release machinery (Gingrich and Byrne, 1985; Hochner et al., 1986). 5-HT can increase the rate of spontaneous release at cultured *Aplysia* sensory-motor synapses by a mechanism that seems to be independent of changes in the presynaptic levels of Ca^{2+}. This modulation of the secretory mechanism underlying spontaneous release may reflect activation of the second process by 5-HT (Dale and Kandel, 1990). While cAMP can trigger this second process a further second messenger pathway may also be involved: possibly, protein kinase C (PKC). We show here that in *Aplysia* saline, containing zero Ca^{2+}, activation of PKC by application of phorbol dibutyrate (PDBu, 1-50 nM) to *Aplysia* sensory-motor synapses in culture increases the occurrence of mEPSPs in a dose dependent manner (120 nM PDBu caused five-fold increase in the frequency of mEPSPs) without altering the amplitude of the mEPSPs. The inactive isomer, α-PDBu, had no effect. Bath application of the generalized kinase inhibitor H-7 (200 μM) partially reversed the enhancement of spontaneous release by both 5-HT and PDBu. This suggests that activation of PKC may contribute to the Ca^{2+}-independent enhancement of spontaneous release that accompanies the effects of 5-HT. Therefore, PKC may contribute along with cAMP-dependent kinase to a direct modulation of secretion that constitute the second process of presynaptic facilitation.

419.2

ACTIVITY-DEPENDENT MODULATION OF SPONTANEOUS TRANSMITTER RELEASE IN SINGLE CULTURED *APLYSIA* SENSORIMOTOR NEURON SYNAPSES. L.S. Eliot, E.R. Kandel and R.D. Hawkins. Ctr. Neurobiol. & Behav., Columbia Univ., NYSPI, & HHMI, NY, NY 10032.

An *Aplysia* L7 motor neuron co-cultured with a single presynaptic sensory cell exhibits spontaneous miniature EPSPs which can be used to assay the release process directly, independent of the action potential (Dale and Kandel, 1990). Cultured sensorimotor synapses undergo homosynaptic depression with low frequency stimulation (< 1 Hz), PTP with high frequency stimulation (20 Hz for 2s), and longer-lasting facilitation when tetanus is given temporally paired, but not when it is given unpaired with 5-HT. We have begun to test whether there are changes in spontaneous release during these various activity-dependent forms of synaptic plasticity, as might be predicted if they involve depletion or mobilization of synaptic vesicles (Gingrich and Byrne, 1985). We have found that tetanus increases the frequency of spontaneous mEPSPs in parallel with facilitation of the evoked EPSP. In five experiments, the frequency of release increased from a baseline of .050 ± .003 s^{-1} to .775 ± .085 s^{-1} in the 1-min. interval following tetanus and remained significantly elevated 7 min after tetanus (.109 ± .024 s^{-1}; $p < .01$, Dunnett's multiple range test), but not 12 min after tetanus. Similarly, the evoked EPSP was significantly facilitated 5 and 10 min after the tetanus (163 ± 20 and 146 ± 20%, respectively; $p < .01$) but not at 15 min post-tetanus. Preliminary experiments show a large increase in spontaneous release following both paired (5-HT + tetanus) and unpaired training. But unlike facilitation of the evoked EPSP, which is pairing-specific, the increase in spontaneous release appears not to be pairing-specific, suggesting that this component of mobilization does not contribute to the pairing-specific facilitation.

419.3

PRESYNAPTIC INHIBITION INDUCED BY G PROTEIN ACTIVATION AT THE SQUID GIANT SYNAPSE. S.D. Hess and G.J. Augustine. Univ. of Southern California, Los Angeles, CA 90089 and Max Planck Institut, Goettingen, FRG.

Guanine nucleotides were microinjected directly into giant presynaptic terminals of squid (Loligo pealei and L. opalescens) to examine the possible role of G proteins in transmitter release. Injection of GTP-gamma-S irreversibly reduced postsynaptic currents (PSCs) evoked by presynaptic action potentials by 70 to 85% (n=18). In contrast, injection of GTP caused a much smaller and reversible reduction (25%, n=10). GDP-beta-S also reduced transmitter release by approximately 40% (n=3). GTP-gamma-S depolarized the presynaptic terminal 9.9 mV and decreased the action potential amplitude, while GTP had little effect on presynaptic potentials. When injected into voltage-clamped terminals held at -70 mV, GTP-gamma-S reduced transmitter release by only 34.5% (n=2). These results suggest that activation of one or more presynaptic G proteins by GTP-gamma-S inhibits transmitter release. One component of this inhibition is caused by a decrease in the presynaptic membrane and action potentials. Supported by NIH NS21624, NS08392 and MPI funds.

419.4

HISTAMINE STIMULATES SYNAPSIN II PHOSPHORYLATION AND CATECHOLAMINE RELEASE IN BOVINE ADRENAL MEDULLARY CHROMAFFIN CELLS. J.A.Firestone, M.D.Browning. *Dept. of Pharmacology, Univ. of Colorado Hlth. Sci. Cntr., Denver, CO 80262.*

Adrenal medullary chromaffin cells have classically been used as a model system for the study of neurotransmitter release. Activation of nicotinic receptors stimulates catecholamine (CA) release from primary cultures of bovine chromaffin cells. Histamine also stimulates CA release from these cells in a time and dose-dependent manner. We have shown (Haycock *et al.*, J. Neurosci., 8, 3233, 1988) that nicotine stimulates incorporation of ^{32}P into synapsin II (a vesicle-associated phosphoprotein thought to play a key role in neurotransmitter release). This phosphorylation is closely correlated with nicotine-stimulated release of CA. We hypothesized that histamine would also stimulate an increase in synapsin II phosphorylation paralleling its effect on secretion. We report here that histamine stimulates ^{32}P-incorporation into synapsin II. The time course of histamine-stimulated phosphorylation of synapsin II closely parallels the histamine-stimulated release of CA. The magnitude of histamine-stimulated secretion is less than that stimulated by nicotine. Interestingly, the extent of histamine-stimulated phosphorylation of synapsin II is also less than for nicotine. After two minutes of treatment, histamine-stimulated phosphorylation of synapsin II is 40% of that produced by nicotine, while release stimulated by histamine is 30% of the release stimulated by nicotine. Thus, there is a strong correlation between synapsin II phosphorylation and CA release in response to two distinct secretogogues. One-dimensional phosphopeptide maps of synapsin II yielded a characteristic 18kD phosphopeptide. This portion of the molecule is known to contain the site phosphorylated *in vitro* by the cAMP-dependent protein kinase and by Ca2+/calmodulin kinase I. The determination of the kinase which is active in the present *in situ* analyses is the subject of current investigation. The data presented here suggest that synapsin II phosphorylation may play a role in CA release from bovine adrenal medullary chromaffin cells.

419.5

IMAGING CALCIUM TRANSIENTS IN PATCH CLAMPED CHROMAFFIN CELLS. G.J. Augustine and E. Neher. Max Planck Inst. for Biophysical Chemistry, Goettingen, FRG.

We have used video microscopy to examine the intracellular Ca concentration ($[Ca]_i$) changes produced in single, cultured chromaffin cells by depolarization. Brief (20-2000 ms) depolarizing pulses, applied via a patch pipette, caused measurable changes in the fluorescence of fura-2 (0.1-0.5 mM) when the depolarizations were within the voltage range for activation of Ca channels (ca. -20 to +50 mV). These signals were due to changes in $[Ca]_i$ because they were abolished by inclusion of 10 mM EGTA in the internal dialysis solution. The changes in $[Ca]_i$ had a characteristic spatio-temporal pattern: when the Ca channels were open, $[Ca]_i$ changes were largest (nominally up to 0.5 uM) in the region just beneath the plasma membrane and smallest in the central cytoplasm of the cell. Closure of the Ca channels, either by inactivation or repolarization of the membrane potential, caused the spatial gradients to collapse very quickly (half-time 200 ms or less). These results indicate that Ca channels in the plasma membrane are the primary source of the $[Ca]_i$ transient produced by depolarization and that Ca (and/or Ca-loaded fura-2) rapidly diffuses into the center of the cell. Transient $[Ca]_i$ gradients may play an important role in these cells during depolarization-induced secretion.

419.6

ROLE OF ACTION POTENTIAL BROADENING AND K CURRENT INACTIVATION IN THE FREQUENCY-DEPENDENT FACILITATION OF CALCIUM ENTRY INTO PITUITARY NERVE TERMINALS. M.B. Jackson, G.J. Augustine & A. Konnerth. Dept. Physiology, Univ. Wisconsin, Madison, WI and Max Planck Inst. for Biophysical Chemistry, Goettingen, FRG,

We have made simultaneous patch clamp and fura-2 $[Ca]_i$ measurements from nerve terminals in thin slices of rat neurohypophysis. Trains of action potentials (APs) evoked at 20 Hz caused large increases in $[Ca]_i$ while evoking the same number of APs at 1 Hz produced much smaller changes in $[Ca]_i$. High frequency stimulation also broadened APs. Broadening of APs by another procedure, application of 2 mM TEA, increased the $[Ca]_i$ signals evoked by low frequency stimulation. Furthermore, $[Ca]_i$ changes measured in voltage clamped terminals were found to be a sensitive function of the duration of depolarizing voltage pulses. Voltage clamp measurements revealed two separable components of voltage-activated K^+ current: one which inactivated by 50% in approximately 50 ms at +50 mV and another which did not decline over 0.5 s. A series of brief, AP-like pulses selectively reduced the inactivating component of K^+ current. A plausible explanation for these results is that a frequency-dependent decline in the inactivating K^+ current broadens the presynaptic AP which, in turn, facilitates Ca entry. This presumably causes the frequency-dependent facilitation of AP-evoked hormone release reported for these terminals.

419.7

DEPOLARIZATION INDUCED CHANGES IN INTRACELLULAR FREE CALCIUM IN INDIVIDUAL NERVE ENDINGS FROM THE NEUROHYPOPHYSIS. E. Stuenkel, Dept. of Physiology, Univ. of Michigan, Ann Arbor, MI 48109.

Although neurotransmitter or neurohormone release is dependent on depolarization-induced changes in intraterminal free $[Ca^{2+}]$ anatomical limitations of mammalian nerve terminals have precluded direct monitoring and characterization of such changes at single axon endings. Using axon terminals isolated from the neurohypophysis of the rat, changes in $[Ca^{2+}]_i$ in individual endings were examined in response to depolarizing stimuli. $[Ca^{2+}]_i$ was monitored by dual wavelength microspectrofluorometry of cytoplasmic fura-2. Induction of membrane depolarization by elevated concentrations of K^+_o led to a rapid, dose-dependent (EC_{50} = 35 mM) increase in $[Ca^{2+}]_i$ (150 nM increase at EC_{50}) that was sensitive to block by the dihydropyridine (DHP) nicardipine (IC_{50} = 2μM), D888 and by the inorganic Ca^{2+} channel blockers Cd^{2+} and La^{3+}. ω-conotoxin GVIA was without effect on the K^+-induced increase in $[Ca^{2+}]_i$. Depolarization of the nerve endings by veratridine resulted in a similar rapid, DHP- and D888-sensitive, increase in $[Ca^{2+}]_i$. Close correlation was found between the K^+-induced increase in $[Ca^{2+}]_i$ and reported values of vasopressin secretion obtained on populations of isolated terminals. K^+-induced increases in $[Ca^{2+}]_i$ were maintained throughout a sustained depolarizing stimulus and were dependent on sustained influx of Ca^{2+} via DHP-sensitive Ca^{2+} channels. Recovery to basal $[Ca^{2+}]_i$ followed removal of the depolarizing stimulus with the rate of recovery being dependent on the duration of the depolarizing stimulus. Recovery of $[Ca^{2+}]_i$ was partially blocked by metabolic inhibitors and La^{3+} but was unaffected by removal of extracellular Na^+ or application of vanadate thereby suggesting a dominant role for Ca^{2+}-ATPase activity over Na^+/Ca^{2+} exchange in recovery.

419.8

A Comparison of the Subsecond Kinetics of ^{3}H-Glutamate and Endogenous Glutamate Release from Neurons. T.J. Turner and K. Dunlap, Dept of Physiology, Tufts University School of Medicine, Boston, MA 02111.

The aim of this study is to characterize the release of "fast" transmitters, such as glutamate (glu), as well as slow, peptidergic transmitters, like Substance P (SP), from intact neurons on a time scale approaching that on which synaptic transmission occurs. To accomplish this, we are using a superfusion device with 50ms time resolution to stimulate release from sensory neurons cultured from embryonic chick dorsal root ganglia; such neurons are capable of releasing both glu and SP. The neurons were grown on collagen-coated cellulose acetate filters (i.e., Millicell units) for 7-14d. The neurons concentrate ^{3}H-glu when incubated with the isotope (10^{-6}M) for 10-20 minutes prior to superfusion. Depolarizing the neurons with K^+ (60-150 mM) increased the rate of isotope release up to 5-fold, but no calcium-dependent release of ^{3}H-glu from these neurons could be observed.

To determine whether the lack of calcium-dependent release of ^{3}H-glu was due to the inability of the isotope to penetrate the vesicular release pool in these neurons, we developed a biochemical assay to measure <1 pMole of endogenous glu. Using rat cortical synaptosomes as a starting point, we found that the subsecond kinetics of ^{3}H-glu release was similar or identical to release of ^{3}H-GABA as characterized previously (Turner and Goldin [1989], Biochem **28,** 586). Preliminary results suggest that the kinetics of endogenous glu release from synaptosomes is similar to that for ^{3}H-glu. The relationship between endogenous and isotopic release from cultured neurons remains to be determined.

419.9

$GABA_A$ AND $GABA_B$ AUTORECEPTOR REGULATION OF GABA RELEASE IN THE VENTRAL PALLIDUM (VP): A STUDY USING MICRODIALYSIS IN FREELY MOVING RATS. A.J. Bourdelais and P.W. Kalivas. Dept. of VCAPP, Wash. St. Univ., Pullman, WA 99164.

There is evidence that presynaptic GABA receptors modulate GABA release in the CNS. It has been demonstrated that the $GABA_A$ agonist muscimol was able to inhibit GABA release from synaptosomes obtained from the substantia nigra pars compacta, whereas the $GABA_B$ agonist baclofen was able to inhibit GABA release from synaptosomes obtained from the substantia nigra pars reticulata (Pittaluga, Eur. J. Pharm., 144:45-52, 1987). We used microdialysis to demonstrate autoreceptor regulation of GABA release in the VP. Rats were chronically implanted with a guide cannula 3 mm above the VP. Post-surgery (7-8 days) the rat was placed in the dialysis box and a dialysis probe was inserted into the VP via the guide cannula. After a 3 hr equilibration period, 5x20 min baseline samples were collected and then dialysis buffers changed to one of the following stepwise increases in agonist concentration: a) dialysis buffer, b) dialysis buffer + 0.1 μM, 1.0 μM, and 10.0 μM muscimol, or c) dialysis buffer + 1.0 μM, 10.0 μM, and 100.0 μM baclofen. Each concentration of agonist was perfused for 80 min; samples were collected every 20 min. Artificial CSF, at a flow rate of 2.13 μl/min was used for dialysis. HPLC-EC was used to measure GABA levels, with OPA/t-butylthiol precolumn derivitization. Baclofen (10.0 μM and 100.0 μM) decreased extracellular GABA levels by 25% and 35%, respectively, as compared to baseline. Muscimol, however, increased extracellular GABA levels by 50% at 1.0 μM, as compared to baseline. No changes in GABA concentration were seen at the other concentrations of muscimol.

419.10

• THE ROLE OF PRESYNAPTIC POTASSIUM CHANNELS IN THE MODULATION OF ACETYLCHOLINE RELEASE FROM HIPPOCAMPAL SLICES. • **C.G. Benishin Dept. of Physiology, University of Alberta Fac. of Med., Edmonton, Alberta T6G 2H7.**

Subcellular mechanisms involved in the modulation of acetycholine (ACh) release in the central nervous system are still not clearly understood. The objective of these studies was to examine the role of presynaptic K channels in the presynaptic modulation of ACh release in the hippocampus. Modulation of ACh release from hippocampal slices was examined in a perfusion system, with the release of ACh stimulated by exposure of slices to elevated K^+ concentration. Under these conditions, evoked release is not sensitive to inhibition by tetrodotoxin. ACh release under these conditions could be inhibited in a dose-dependent manner by a muscarinic agonist (oxotremorine, Oxo) and a purinergic agonist (2-chloroadenosine, 2-ClAdo). α-dendrotoxin (α-DaTx), which was previously shown to selectively block voltage-gated inactivating K channels in nerve endings, did not effect the release of ACh under resting or depolarized conditions. However, α-DaTx reversed the 2-ClAdo induced inhibition of release, but did not alter the Oxo induced inhibition. These results suggest that a α-DaTx sensitive K channel may be activated as an obligatory step in the modulation of ACh release by presynaptic purinergic receptor activation, but not in the modulation by presynaptic muscarinic receptors.

419.11

EVIDENCE THAT TETRAETHYLAMMONIUM-SENSITIVE K+ CHANNELS CONTRIBUTE TO PRESYNAPTIC SPIKE REPOLARIZATION AND CONTROL OF TRANSMITTER RELEASE IN HIPPOCAMPAL SLICES.
O. Paulsen, M. Raastad and J.F. Storm.
Inst. of Neurophysiology, Karl Johans gt. 47, 0162 Oslo, Norway.

Presynaptic K channels can indirectly control transmitter release by determining spike duration and hence Ca influx. In the hippocampus, the K channel blocker 4-aminopyridine broadens the presynaptic fiber volley and increases synaptic potentials (Haas et al., Experientia 1983;39:114). It has also been suggested that a TEA-sensitive Ca-activated K current could contribute to presynaptic spike repolarization, thus exerting a feed-back control of Ca influx and transmitter release (Storm, Brain Res. 1987;435:387). To test this and related ideas, we have started to measure the effects of K and Ca channel blockers on the presynaptic fiber volley and the excitatory post-synaptic potential (EPSP) in the CA1 field of rat hippocampal slices.

In submerged slices (23-30 °C) stimulation of CA1 str. radiatum fibers produced field potentials recorded in the same layer. The response consisted of a fiber volley (amplitude 0.2-1.2 mV, duration 1.2-1.8 ms) followed by a field EPSP (0.3-1 mV). The EPSP could be blocked by kynurenic acid or the Ca channel blockers Mn and Cd. The remaining fiber volley could be blocked by TTX. 1 mM tetraethylammonium (TEA) reversibly increased both the EPSP amplitude (by 17-100%; n=12) and the fiber volley duration (5-15%; n=12). The EPSP increase was also observed intracellularly (n=8). The broadening of the fiber volley survived blockade of the EPSP by kynurenic acid (n=3), whereas Ca-free medium with 2 mM Mn seemed to occlude the effect of TEA.

These data suggest a role for TEA-sensitive K channels in presynaptic spike repolarization and the control of transmitter release.

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419.12

ALPHA ADRENOCEPTOR ACTIVATION INHIBITS THE EARLY BUT NOT THE LATE IPSP IN AREA CA1 OF THE RAT HIPPOCAMPUS WHILE INCREASING THE SPONTANEOUS RELEASE OF GABA FROM PRESYNAPTIC INHIBITORY INTERNEURON TERMINALS.
V.A. Doze, G.A. Cohen, K.D. Parfitt and D.V. Madison. *Department of Molecular & Cellular Physiology, Stanford Univ. School of Medicine, Stanford, California 94305.*

Previous work has shown that alpha adrenoceptor activation decreases the evoked inhibitory postsynaptic potential (IPSP) in area CA1 of the rat hippocampus. Using intracellular recordings in slices treated with agents that selectively block GABA-A (picrotoxin-100μM) or GABA-B (saclofen-100μM) receptor mediated responses, we examined the effect of epinephrine (EPI) on isolated early IPSP's and late IPSP's. We found that EPI-1μM potently decreases the amplitude of the early IPSP (>50%) without affecting the size of the late IPSP. To further examine this mechanism, we used whole cell voltage-clamp recordings in slices treated with tetrodotoxin (1μM) to detect spontaneous release of GABA from the inhibitory interneuron terminals (IPSC's). We found that EPI caused an increase in both the frequency and amplitude of the spontaneous IPSC's. Our results suggest that EPI decreases evoked synaptic inhibition while increasing the spontaneous quantal release of GABA from interneuron terminals. We speculate that depolarization of the presynaptic interneuron terminal may be the mechanism for these seemingly paradoxical actions of EPI on evoked versus spontaneous synaptic inhibition. In addition to the above findings, we found that the action of EPI on the evoked IPSP was strongly dependent on extracellular potassium levels $[K^+]_o$. When $[K^+]_o$ was lower than 3mM, EPI was ineffective. However, when $[K^+]_o$ was 5mM or above, EPI potently reduced the evoked IPSP. Similar findings were obtained with selective alpha adrenoceptor agonists (6-fluoroEPI), but not beta adrenoceptor agonists (isoproterenol). Thus, alpha adrenoceptor activation may have profound significance during epileptiform activity when $[K^+]_o$ rises to 5mM and above.

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419.13

PHARMACOLOGICAL AND FUNCTIONAL CHARACTERIZATION OF AN ALPHA ADRENOCEPTOR IN AREA CA1 OF THE RAT HIPPOCAMPUS.

D.V. Madison and V.A. Doze. *Department of Molecular and Cellular Physiology, Stanford University School of Medicine, Stanford, California 94305.*

Previous work has shown that norepinephrine (NE) decreases synaptic inhibition in the rat hippocampus via activation of an alpha adrenoceptor. To further characterize the subtype of alpha adrenoceptor involved in mediating this action, we examined the actions of a large number of known alpha adrenoceptor agonists on the size of the evoked inhibitory postsynaptic potential. We found that this is an alpha adrenoceptor of unusual pharmacology. This particular adrenoceptor is activated by phenethylamines with a rank order as follows: 6-fluoroepinephrine > epinephrine ≥ 6-fluoronorepinephrine > NE > phenylephrine > isoproterenol. The selective alpha-2 agonist α-methyl-NE also possessed significant activity at this adrenoceptor. Initial experiments with antagonists selective for alpha-1 (prazosin, corynanthine) and alpha-2 (rauwolscine, yohimbine) adrenoceptors suggest that the predominant subtype of adrenoceptor involved is an alpha-2. Interestingly, compounds of either the guanidinum (e.g., guanabenz, guanfacine) or imidazoline class (e.g., cirazoline, oxymetazoline, clonidine, para-amino-clonidine) were either ineffective or only weakly active (partial agonists) against this adrenoceptor. These results are in agreement with several recently published studies indicating that the imidazolines and phenethylamines interact differently with alpha adrenoceptors, and that many actions of the imidazolines previously ascribed to alpha adrenoceptors may be attributable to a newly discovered imidazoline/guanidinium receptive site distinct from alpha adrenoceptors. In summary, our results confirm that an alpha adrenoceptor mediates the disinhibitory action of NE in the rat hippocampus and suggest that this adrenoceptor is of the alpha-2 subtype. We have found, however, that only catechol-type compounds of the phenethylamine class behave as full agonists at this particular alpha adrenoceptor.

D.V.M. is a Lucille P. Markey Scholar and this work was supported in part by a grant from the Lucille P. Markey Charitable Trust. V.A.D. is supported by the NIMH. The authors acknowledge the gift of 6-fluoroepinephrine from K. L. Kirk (NIDDK).

419.14

LONG TERM DEPRESSION OF EXCITATORY SYNAPTIC TRANSMISSION IN THE NEONATAL RAT SPINAL CORD.

A. Lev-Tov, M. Pinco* and R. Lavy*. Dept. of Anatomy, The Hebrew Univ. Medical School, Jerusalem 91010, Israel.

Synaptic transmission was studied in the isolated spinal cords of neonatal rats (age 6-10 days) using intracellular and whole cell patch-clamp recordings of excitatory post synaptic potentials (EPSPs) and currents (EPSCs), and extracellular recordings of monosynaptic reflexes. The EPSPs and monosynaptic reflexes, were severely depressed at stimulation rates above 0.1 Hz. The depression disappeared only as the interpulse intervals exceeded 50 seconds. Partial recovery from the depression was observed during repetitive stimulation at 0.5-2 Hz, usually following the first 100-200 stimuli. The reduction in the EPSP amplitude was not accompanied by any detectable changes in the time courses of the EPSPs, indicating that the long-term depression is of presynaptic nature. Extracellular recordings of synaptic fields generated in the motor nucleus by dorsal root stimulation, revealed that the terminal potentials preceding the synaptic fields were not affected by the frequency of stimulation. We therefore suggest, that the long term depression of synaptic transmission in the neonatal rat spinal cord, reflects inability of the premature transmitter release machinery to maintain high release levels from fully invaded afferent terminals at low activation frequencies.

419.15

NH_4^+ DECREASES EXCITATORY SYNAPTIC TRANSMISSION BETWEEN CEREBELLAR NEURONS IN VITRO. W. Raabe. Depts. Neurology, VA Med. Ctr. and Univ. of Minnesota, Minneapolis, MN, 55417.

Glutamine is presumed to be a precursor for glutamate used as transmitter. Glutaminase catalyzes the cleavage of glutamine to glutamate. NH_4^+ inhibits glutaminase. To investigate the role of glutamine as precursor for glutamate this study examines the effects of NH_4^+ on glutamatergic excitatory synaptic transmission.

Wole cell patch voltage clamp recordings were obtained from large cerebellar neurons (Purkinje cells) in primary dissociated tissue culture. Stimulation of nearby neurons with an extracellular electrode produced monosynaptic excitatory currents as identified by their ability to follow stimulation at 20 Hz. NH_4^+ (10 mM) reversibly abolished evoked excitatory postsynaptic currents (EPSCs) but did not abolish spontaneous miniature EPSCs. TTX (5 μM) and Mg^{++} (10 mM) also abolished evoked EPSCs and did not affect spontaneous miniature EPSCs, indicating that the latter are due to spontaneous release of transmitter from presynaptic terminals.

The lack of an effect of NH_4^+ on spontaneous transmitter release indicates that abolition of evoked EPSCs by NH_4^+ is not due to interference with glutamine as a precursor for glutamate. Possibly, NH_4^+ abolishes evoked EPSCs by blocking the invasion of action potentials into presynaptic terminals as it has been shown for Ia-afferents in cat spinal cord (J. Neurophysiol. 62: 1461, 1989).

ION CHANNELS: LIGAND-GATED I

420.1

ADDITION OF NERVE OR NERVE CONDITIONED MEDIUM TO CULTURED XENOPUS MYOCYTES ALTERS ACETYLCHOLINE RECEPTOR CHANNEL POPULATION AND KINETICS

J. Rohrbough* and Y. Kidokoro. Dept. of Physiology, U.C.L.A. School of Medicine, Los Angeles CA., 90024.

Single-channel recordings of acetylcholine receptor (AChR) channels were taken to assess channel population and kinetics after the addition of nerve or nerve-conditioned medium to *Xenopus* myocytes cultured alone for several days. Muscle cultures were prepared from stage 16-18 embryos, and stage 22-23 neural tubes or nerve-conditioned medium was added after 4-5 days in culture.

It was previously found that the addition of nerve to *Xenopus* myocyte cultures had the surprising effect of increasing mean AChR channel open time in noise analysis (Brehm et al. *Dev. Biol.* **91**, 1982), indicating an increase in the relative number of low conductance (low-g) channels. We verified this effect, measuring the percentage of high conductance (high-g) single channel events after 6 days in culture. In control 6-day cultures, the percentage of high-g events was 54%. The percentage decreased to 36% and 33% when neural tube cells were added on day 5 or day 4, respectively. The effect was similar in both nerve contacted and non-contacted cells. Similarly, after one day exposure to nerve-conditioned medium, the percentage of high-g events decreased to 39%. These results suggest the possible release of factor(s) by the nerve which alters the synthesis rate of the low-g receptor. A remaining question we are addressing is whether the kinetics of either the existing or of the new receptor population are altered after exposure to nerve. In aneurally cultured *Xenopus* myocytes, channel burst duration, particularly for the low-g channel, decreases during development. After 6 days, most low-g burst duration histograms have an excess of brief events but are otherwise fit by a single exponential. Preliminary results suggest that in many cases, low-g burst duration histograms are markedly double exponential, with a larger slow component, after nerve is added.

420.2

EPSILON SUBUNIT CONFERS BRIEF CHANNEL OPEN TIME ON MULTIPLE FORMS OF MUSCLE ACH RECEPTOR. Y. Liu, P. Camacho, G. Mandel*, and P. Brehm. Dept. of Neurobiology & Behavior, SUNY at Stony Brook, Stony Brook, NY., 11794.

The role of the epsilon subunit in determining ACh receptor channel function was tested by co-expressing rat epsilon RNA with various combinations of mouse α,β,γ and δ RNA. Injection of αβδγ RNA into *Xenopus* oocytes resulted in several conductance classes, all bearing long channel open time whereas injection of αβδε RNA led to multiple channel types all bearing brief open time. We tested the possibility that functionally distinct forms of ε containing receptors resulted from differences in subunit composition by injecting specific combinations of subunit RNAs. In the combinations of two subunits tested with 10μM ACh, only αε showed reasonable expression with αδ, αβ, and αγ expressing little or no current. However, all of the combinations containing 3 subunits tested, αβγ, αβδ, αδγ, and αδε expressed functional receptors, with αδε exhibiting the largest currents (1-10μA). Single channel recordings from αδε channels revealed a single amplitude class. The events corresponded to the intermediate conductance class observed with the full complement of αβδε on the basis of similarities in both open time and conductance. Our findings indicate that inclusion of ε subunit in the formation of functional receptors results in brief open times characteristic of receptors on adult skeletal muscle. The finding that the epsilon confers brief open times to ACh receptors of different subunit composition suggests that this subunit differs from α, β, γ and δ in that it plays an active role in shortening channel open time of ACh receptors. (Supported by NIH grant 18205 to PB).

420.3

NICOTINIC ACh RECEPTORS OF INSECTS AND VERTEBRATES: ACTIONS OF A BISQUATERNARY AMMONIUM SERIES.

C.A. Leech*, S.C.R. Lummis*, C.W. Holyoke and D.B. Sattelle. AFRC Laboratory of Molecular Signalling, Department of Zoology, Cambridge CB2 3EJ, U.K. and E. I. du Pont, Agricultural Products Department, Wilmington, DE 19880

Patch-clamp studies on dissociated neurones of housefly (*Musca domestica*) and cockroach (*Periplaneta americana*) have revealed 3 distinct conductances in the presence of acetylcholine (20pS; 30-40pS; 60-80pS), of which the 30-40pS channel is the most common. Open times are best fitted by 2 exponential components. Closed time distributions show greater variation and require 2-4 exponential fits. Voltage-clamp studies on identified cockroach neurones have characterized a nicotinic receptor blocked by α-bungarotoxin, k-bungarotoxin, dihydro-β-erythroidine and lophotoxin. Displacement of [125I] α-bungarotoxin binding by a C4-C12 bisquaternary ammonium series has shown that nicotinic receptors of cockroach CNS, chick CNS and chick muscle respond differently to an increase in molecular size of these compounds. Whereas in chick muscle, the percent inhibition increases with separation of the quaternary nitrogens, brain inhibition is greatest at the two extremes. The cockroach receptor exhibits some of the properties of both classes of vertebrate nicotinic receptors in its response to the C4-C12 compounds.

420.4

[^{3}H]-NICOTINE PHOTOAFFINITY LABELS TYR-198 IN THE ALPHA-SUBUNIT OF THE *TORPEDO* NICOTINIC ACETYLCHOLINE RECEPTOR.

R.E. MIDDLETON AND J.B. COHEN. Dept. of Anatomy and Neurobiology, Washington University, St Louis, MO 63110.

The agonist [^{3}H]-nicotine binds at equilibrium to the acetylcholine receptor with Kd=0.5 μM. We have studied by SDS-PAGE the photoincorporation of [^{3}H]-nicotine into polypeptides of *Torpedo* postsynaptic membranes. Irradiation at 254 nm resulted in labeling of α- and γ-subunits that was inhibited by agonists and competitive antagonists but not by noncompetitive antagonists. The amplitude of specific labeling varied with the [^{3}H]-nicotine concentration (K=1.5 μM), with maximal incorporation of ≈0.5% (α) and ≈0.1% (γ). Inhibition of the γ-subunit labeling by d-tubocurare (d-TC) was consistent with inhibition via a single high affinity site (IC_{50}=100 nM) while inhibition of the α-subunit labeling by d-TC was fit by a two site model (IC_{50}=100nM and 1.0 μM). This suggests that the agonist site that binds d-TC with high affinity is located at the interface of the α- and γ-subunits and that each α-subunit is labeled by [^{3}H]-nicotine.

The specific photoincorporation into the α-subunit was localized to a 20 Kd proteolytic fragment which begins at ser-173 and contains the amino acids known to be labeled by competitive antagonist affinity labels. Sequential Edman degradation of this fragment indicated that tyr-190, cys-192 and tyr-198 were the principal sites of incorporation. Analysis of α-subunit peptides generated by chymotrypsin established that tyr-198 contains ≈40% while tyr-190 and cys-192 each contained ≈10 to 20% of the specifically incorporated [^{3}H]-nicotine. Since competitive antagonist affinity reagents label principally tyr-190 and cys-192, the labeling of tyr-198 identifies an adjacent amino acid important for agonist binding.

420.5

BOTH ALPHA AND BETA SUBUNITS CONTRIBUTE TO THE AGONIST SENSITIVITY OF NEURONAL NICOTINIC ACETYLCHOLINE RECEPTORS.

C. W. Luetje and J. Patrick. Division of Neuroscience, Baylor College of Medicine, Houston TX 77030.

A family of genes has been identified which encode subunits of nicotinic acetylcholine receptors (nAChR) and are expressed in the nervous system. The α2, α3, and α4 subunits can each form functional nAChR when expressed in *Xenopus* oocytes in pairwise combination with either the β2 or β4 subunits. We find that the agonist pharmacology of mouse muscle nAChR (α1β1γδ) expressed in oocytes matches that of the nAChR expressing mouse muscle cell line BC3H1, demonstrating the accuracy of the oocyte expression system in the study of ligand gated ion channel pharmacology. Each of six neuronal subunit combinations displays an unique rank order of sensitivity to four nicotinic agonists. The α2β2 combination is 3-10 fold more sensitive to nicotine than to acetylcholine, while the α3β2 combination is 10-30 fold less sensitive to nicotine than to acetylcholine and the α3β4 combination is equally sensitive to both nicotine and acetylcholine. nAChR composed of α2, α3 or α4 in combination with β2 are 10-100 fold less sensitive to cytisine than to acetylcholine. In contrast, nAChR composed of α2, α3 or α4 in combination with β4 are 3-10 fold more sensitive to cytisine than to acetylcholine. The α2β2, α3β2 and α3β4 combinations are each equally sensitive to dimethyl-phenylpiperazinium (DMPP) and acetylcholine, while the α2β4, α4β2 and α4β4 combinations are 3-30 fold less sensitive to DMPP than to acetylcholine. Our results demonstrate that the sensitivity of neuronal nAChR to various agonists is dependent not only upon which α subunit, but also upon which β subunit forms the receptor.

420.6

cDNAs AND MONOCLONAL ANTIBODIES DEFINE SUBTYPES OF NEURONAL α-BUNGAROTOXIN-BINDING PROTEINS.

R. Schoepfer, W.G. Conroy,* P. Whiting and J. Lindstrom. The Salk Institute, P.O. Box 85800, San Diego, CA 92138.

Neuronal α-bungarotoxin-binding proteins (αBgtBPs) are homologous, yet different from neuronal and muscle nicotinic acetylcholine receptors (AChRs). Although neuronal αBgtBPs have been detected for almost 20 years, their function is still unknown.

Recently we cloned cDNAs coding for two subunits of chicken brain αBgtBPs. The deduced amino acid sequences define αBgtBPs as members of the AChR gene family and predict that αBgtBPs have cation channels. Subunit-specific monoclonal antibodies (mAbs) were obtained by using bacterially overexpressed, unique subunit segments as immunogens. These mAbs were used to probe western blots of affinity-purified αBgtBPs, neuronal AChRs, and muscle-type AChRs. The results demonstrate the specificity of the mAbs within this gene family and suggest that αBgtBPs and AChR subunits are not coassembled *in vivo*, despite their homology and partial colocalization.

As revealed by immunoprecipitation, the αBgtBP α1 subunit is found in ~90% of all high-affinity (nM) αBgtBPs in embryonic day 18 chicken brains. The α2 subunit is found in ~15% of all αBgtBPs. Further data imply that the α2 subunit is always found in αBgtBPs having the α1 subunit. Thus we have defined an αBgtBP subtype I that has α1 but no α2 subunit and accounts for ~75% of αBgtBPs. Subtype II accounts for ~15% and has α1 and α2 subunits. Other uncharacterized subunits are also present.

420.7

ETHANOL ALTERS NICOTINIC RECEPTOR KINETICS: EFFECT OF CONCENTRATION.

M.M. Fróes, Y. Aracava, and E.X. Albuquerque. Lab. Mol. Pharmacol. II, IBCCF, UFRJ, Rio de Janeiro, 21944, RJ, Brazil & Dept. Pharmacol. Exp. Ther., Univ. Maryland Sch. of Med., Baltimore, MD 21201.

Molecular interactions of ethanol (EtOH) with a number of transmitter–gated channels have been extensively studied. In this work, we examined the concentration–dependent EtOH actions on the kinetics of muscle nicotinic acetylcholine receptor (AChR). Single channel currents were recorded from the perijunctional region of isolated frog muscle fibers under cell–attached patch–clamp condition. We tested a wide range of EtOH concentrations (10 μM–1.7 M) on the current evoked by acetylcholine (ACh, 0.4 μM). Many distinct kinetic alterations that showed concentration–, time– and voltage–dependence could be depicted: (*i*) at all concentrations tested, the mean number of flickers per burst was increased; this effect was enhanced by hyperpolarization and was further accentuated by a prolonged drug exposure; (*ii*) EtOH elicited two major effects on the opening probability (P_{open}); at low concentration (10 μM), a marked increase in the P_{open} (up to 6–fold) was observed; at higher doses (100 μM–1.7 M) the brief increase of P_{open} was followed by a significant depression; and (*iii*) EtOH (1.7 M) decreased the channel conductance and induced two kinetically distinct populations of currents: one comprised of rapid events, and another consisting of long–lasting bursts. In addition, EtOH alone (0.6–1.7 M) activated currents that resembled those seen in the presence of both ACh and 1.7 M EtOH. Our data suggest the existence of multiple sites of action for EtOH on protein and/or lipid components of postjunctional membrane. Support: Capes/Finep/ UFRJ–UMAB Mol. Pharmacol. Training Program, and NIH #P50MH44211.

420.8

NICOTINIC ACETYLCHOLINE RECEPTOR (nAChR) ION CHANNEL CURRENTS IN RAT HIPPOCAMPAL NEURONS.

M. Alkondon and E.X. Albuquerque. Dept. Pharmacol. & Exp. Ther., Univ. of Maryland School of Medicine, Baltimore, MD 21201.

Mapping experiments using radiolabelled cholinergic agonists and *in vivo* studies using extracellular recordings indicated that nAChR are present in different parts of the brain. Single–channel studies from our laboratory (Aracava *et al.*, *FEBS Lett.*, 222:63, 1987) have shown the existence of functional nAChR channels in cultured rat hippocampal neurons. Very recently, a cationic channel for nAChR has also been shown in cultured neurons from retinal ganglion and medial habenula nucleus of the rat and from porcine hypophyseal intermediate lobe cells. This study characterizes the nAChR in hippocampal neurons using whole–cell patch clamp recordings. Hippocampal neurons of fetal rats grown in culture for 3–8 weeks were used and agonists were applied to the neuron using a 'U'–tube perfusion system. Acetylcholine (ACh) (50–100 μM) and (+)anatoxin (Antx) (10 μM) elicited inward currents (IC) which grew linearly with hyperpolarization (–10 to –100 mV). At positive membrane potentials, the amplitude of the currents was smaller indicating the occurrence of an outward rectification for these channels. Antx was about 10 times more potent than ACh in activating the hippocampal nAChR. Rapid inactivation of the IC occurred during the continued presence of the agonist, which was more prevalent with Antx than ACh. Responses to Antx were blocked by d–tubocurarine (10–20 μM), dihydro β–erythroidine (20 μM) and mecamylamine (10 μM). These results disclose the presence of a neuronal type nAChR in cultured rat hippocampal neurons and confirm the existence of an excitatory cholinergic function in the hippocampus. Support: US Army Med. Res. & Devel. Comm. Contr. DAMD17–88–C–8119 & NIH Grant NS25296.

420.9

SIMULTANEOUS FAST REMOVAL OF AGONIST AND BLOCKER REVEALS SLOW TRANSITIONS FROM BLOCKED STATES. A.C.S. Costa and E.X. Albuquerque. Dept. Pharmacol. Exp. Ther., Univ. Maryland Sch. Med., Baltimore, MD 21201 & Lab. Mol. Pharmacol. II, IBCCF, UFRJ, Rio de Janeiro, 21944, Brazil.

We have recently shown that 9-amino-1,2,3,4-tetrahydroacridine (THA) and 9-aminoacridine (9-AA) modify NMDA-gated single channel currents, primarily as open channel blockers. We decided to compare the potency of these compounds on NMDA and non-NMDA currents using whole-cell voltage-clamp on cultured rat hippocampal neurons. Admixtures of agonist and drug were applied via a U-shaped tube. This system produced concentration jumps within ≈100 msec. When NMDA and relatively low concentrations of blocker were applied together (<50μM THA and <25μM 9-AA), a reduction in current amplitude was noticed. Increases in THA or 9-AA concentrations further decreased the current amplitude and also started to produce a 2nd current component. This 2nd peak occurs following the total removal of the test solution. We interpreted this 2nd peak as the result of a slow unblock step after the channel blockade. Fast application of APV plus NMDA failed to reproduce this effect. THA and 9-AA also blocked kainate-gated currents but at concentrations about 20 times higher. Moreover, these two compounds were unable to produce the 2nd component in kainate currents. Similar to NMDA currents, we also observed the presence of a 2nd current component in ACh-gated currents. We used the same superfusion system with some well defined blockers of ACh-gated currents and also THA at rat myoballs. The 2nd component was observed when high concentrations of QX-222, dimethylanatoxin, bupivacaine and THA were applied with ACh. These compounds were previously reported to produce slow transitions to blocked (or desensitized) states. SAD-128, reported to act as a pure fast open channel blocker in all concentrations tested, failed to produce the 2nd component. Fast superfusion systems used to apply and remove agonist and blocker may reveal previously hidden slow reaction steps. For example, in the case of QX-222 this approach tells us that return from a long lived blocked state(s) must be at least in part via the open state (a sequential scheme). Support: U.S. Army Med. Res. Devel. Comm. Contract DAMD17-88-C-8119 and FINEP and NIH Grant NS-25296.

420.10

Inhibition of Excitatory Amino Acid Currents by General Anesthetic Agents. Robert W. Peoples, David M. Lovinger, and Forrest F. Weight. Section of Electrophysiology, National Institute on Alcohol Abuse and Alcoholism, Rockville, MD 20852.

Previous studies in this laboratory have shown that ethanol and other aliphatic alcohols inhibit responses mediated by N-methyl D-aspartate (NMDA) receptors in mammalian neurons at physiologically relevant concentrations. We have investigated the actions of various anesthetic agents on excitatory amino acid-activated ion currents in voltage-clamped mouse hippocampal neurons in culture using the whole-cell patch-clamp technique. Pentobarbital inhibited kainate and quisqualate currents with similar potency (IC_{50} ~ 50 μM), but produced a greater maximal inhibition of the quisqualate current (70% inhibition vs. 55% for kainate). Pentobarbital blocked both kainate and quisqualate currents in a use-dependent but rapidly reversible manner, and thus may bind to a site within, or near the opening of, the ion channels. Pentobarbital had essentially no effect on the NMDA current. In contrast, diethyl ether exhibited a pharmacological profile similar to that of ethanol, in that it inhibited the NMDA current to a greater extent than the kainate and quisqualate currents (~ 80% for NMDA vs. ~ 25% for kainate at 80 mM diethyl ether). We are currently investigating actions of other anesthetic agents on excitatory amino acid currents. Inhibition of excitatory amino acid neurotransmission in the CNS by anesthetics could be expected to contribute to their depressant effects on behavior. (R.W.P. is a fellow of the National Research Council.)

420.11

THE PHARMACOLOGY OF CHLORISONDAMINE INVESTIGATED IN RAT BRAIN. P.B.S.Clarke, I.Chaudieu, P.Boksa, and R.Capek. Depts of Pharmacology and Psychiatry, McGill Univ., Montreal, Canada H3G 1Y6.

A single administration of the ganglion blocker chlorisondamine (CHL: 10 mg/kg sc) produces a quasi-irreversible blockade of nicotine's central actions in the rat, of unknown mechanism (Clarke 1984). Expt 1 sought to determine if CHL is acutely neurotoxic; 3 d after CHL (10 mg/kg sc), central effects of nicotine were demonstrably blocked but no neuronal degeneration was detected by the Gallyas method. In Expt 2, CHL (10 mg/kg sc) blocked central effects of nicotine when tested 2 weeks later, but did not significantly alter the B_{max} of high-affinity ^{3}H-nicotine binding in brain. In Expt 3, CHL inhibited ^{3}H-dopamine release induced by NMDA (10^{-5} M) from cultured fetal rat dissociated mesencephalic cells, but only at high concentrations (ED_{50} 0.68 mM), and had no detectable effect on responses to quisqualate (10^{-5} M) and kainate (10^{-4} M). In Expt 4, CHL inhibited NMDA-mediated synaptically-evoked field potentials in adult rat hippocampal slices, but again only at high concentrations (0.1 - 1 mM), and non-NMDA responses were less affected. These results suggest that chlorisondamine's persistent CNS nicotinic blockade is not accompanied by changes in nicotinic receptor density or by neuronal degeneration. In addition, the drug seems to block excitatory amino acid receptors only with low potency. Supported by NIDA and MRC (Canada).

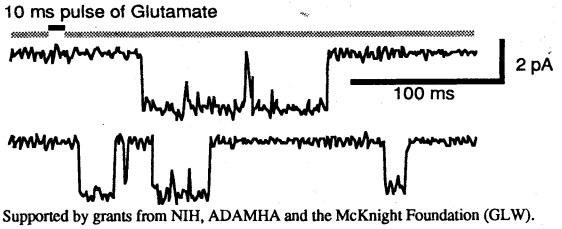
420.12

KINETIC PROPERTIES OF NMDA CHANNELS J. Clements & G. Westbrook, Vollum Institute, Oregon Health Sciences Univ., Portland, OR. 97201

Excitatory synaptic currents in the CNS often exhibit a slow NMDA receptor mediated component which has a rise time of 8-10 ms and a decay time constant of 50-150 ms. To understand the process which produces this slow current, knowledge of the kinetic properties of the NMDA channel is required. Previous studies using whole cell recordings could not produce solution changes in less than 10 ms, precluding detailed study of binding and activation kinetics.

We applied pulses of glutamate to outside-out patches of membrane from cultured hippocampal neurones by rapidly moving the interface of two flowing solutions across the tip of the patch pipette. Control experiments revealed that solution exchange at the surface of the membrane was complete in 1 ms.

Pulses of high concentration glutamate (>100 μM) produced responses with rise times of 8-10 ms, similar to the synaptic current, even when the pulse was as short as 2 ms. This suggests that intrinsic channel kinetics limit NMDA channel activation. At lower concentrations (0.2 to 20 μM), a slower rise was seen. An analysis of the relationship between glutamate concentration and rise time will be presented.



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420.13

CHANNEL KINETICS UNDERLIE NMDA RECEPTOR-MEDIATED SYNAPTIC CURRENTS. R. A. J. Lester, J. D. Clements, G. L. Westbrook and C. E. Jahr. Vollum Institute, Oregon Health Sci. Univ., Portland, OR 97201

In the presence of CNQX (5 μM), long-lasting NMDA receptor-mediated epscs were recorded between pairs of hippocampal neurons in culture using whole cell patch clamp techniques. The competitive NMDA receptor antagonist, D-AP5 (100 μM), did not affect the slow epsc when rapidly applied during its decay. These results suggest that NMDA receptors activated by synaptically released glutamate must remain bound and active for the duration of the slow epsc and thus inaccessible to competitive inhibition. In separate experiments, a 5 ms pulse of L-glutamate (100 μM) to an outside-out membrane patch produced NMDA channel activity that persisted for hundreds of milliseconds indicating that glutamate can remain bound for prolonged periods. The time courses of ensemble averages of these patch currents were very similar to the slow epscs. Both had slow rise times (7.4, epsc; 10. 3, patch response; 10-90 % rise time) and slow decay phases which could be fitted with a double exponential (63 ms, epsc; 87 ms, patch response; first time constant). These results imply that a brief pulse of glutamate in the synaptic cleft is sufficient to account for the slow epsc. The slow rise time of the epsc is due to the activation kinetics of NMDA receptors rather than diffusion of transmitter to extrasynaptic sites. If ligands with different affinities for the NMDA receptor (such as L-aspartate) were released synaptically different duration epscs would result.

Supported by NIH, ADAMHA and the McKnight Endowment Fund for Neuroscience

420.14

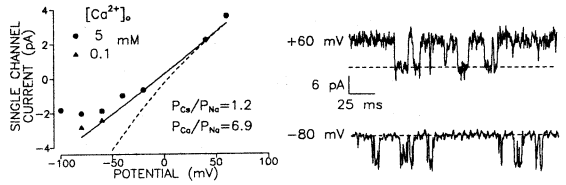
NEUTRAL PEPTIDES CONTAINING THE AMINO ACID SEQUENCE SER-ALA-ALA ACTIVATE A MAGNESIUM-SENSITIVE CATION CHANNEL IN HIPPOCAMPAL NEURONS. J.R. Pace, M.E. Mitchell†, S.M. Paul and M.A. Rogawski. Neuronal Excitability Section, Medical Neurology Branch, NINDS, and Clinical Neuroscience Branch, NIMH, Bethesda, MD 20892 (†HHMI Scholar).

Fast excitation of neurons by conventional neurotransmitters such as glutamate, acetylcholine and serotonin occurs by gating of cation-permeable membrane channels. With the exception of excitatory amino acid receptor-coupled channels which can be activated by high concentrations of certain acidic (glutamate or aspartate containing) peptides, notably *N*-aspartylglutamate, peptide gated cation channels have not previously been described in neurons. In the present study using whole-cell voltage-clamp techniques, we characterized an ionic current in cultured hippocampal neurons that is activated by peptides containing the amino acid sequence Ser-Ala-Ala. Peptides active as agonists of this channel include Ser-Ala-Ala-Ser-Leu-Asn (SAANSLN), the prototypic ligand we used in most of our studies, as well as SAAS and SAA; AASL and all possible dipeptide fragments of SAASLN are inactive. At negative holding potentials, SAASLN rapidly elicited an inward current response whose amplitude increased in a concentration-dependent manner between 1-100 μM. Reversal potential measurements indicated that the channels activated by SAASLN are permeable to Na^+, K^+, Cs^+ and Ca^{2+}, with ionic selectivities P_K/P_{Na}, P_{Cs}/P_{Na} and P_{Ca}/P_{Na} of 0.75, 1.2 and 6.9, respectively. Like the channels activated by the excitatory amino acid antagonist *N*-methyl-D-aspartate (NMDA), these channels are blocked in a voltage-dependent fashion by Mg^{2+}. However, the peptide responses are insensitive to the NMDA-receptor antagonist DL-2-amino-5-phosphonovaleric acid (AP5) or to phencyclidine, a potent steric blocker of NMDA-receptor associated channels. The peptide therefore activates a unique and heretofore unrecognized ligand gated ion channel.

420.15

SINGLE CHANNEL CURRENTS ACTIVATED BY A NEUTRAL PEPTIDE IN RAT HIPPOCAMPAL NEURONS. M.E. Mitchell†, J.R. Pace, S.M. Paul and M.A. Rogawski. Neuronal Excitability Section, Medical Neurology Branch, NINDS, and Clinical Neuroscience Branch, NIMH, Bethesda, MD 20892 (†HHMI Scholar).

We have observed that neutral peptides containing the sequence Ser-Ala-Ala (SAA) can activate a Mg^{2+}-sensitive cation current in cultured rat hippocampal neurons (J.R. Pace *et al.*, this meeting). In the present study, we characterized the single channel currents activated by one of these peptides (Ser-Ala-Ala-Ser-Leu-Asn; SAASLN) in excised (outside-out) membrane patches. Single channel recordings were carried out with patch electrodes filled with (in mM): K-gluconate or CsCl, 145; HEPES, 5; $MgCl_2$, 2; EGTA, 1; $CaCl_2$, 0.1. The bathing medium contained: NaCl, 140; HEPES, 10; KCl, 2.5; $CaCl_2$, 0.1 or 5; phencyclidine (10 μM) was added to eliminate NMDA receptor mediated currents. In the presence of low external Ca^{2+} (0.1 mM), SAASLN activated a 46 pS channel whose nearly linear single-channel current-voltage plot reversed near 0 mV. However, with high external Ca^{2+} (5 mM), the observed single channel amplitudes were smaller at negative potentials than predicted by the Goldman-Hodgkin-Katz equation (dotted line), suggesting that Ca^{2+} can rapidly block the channel.



420.16

LOWER CONCENTRATIONS OF KAINATE ARE REQUIRED TO ACTIVATE LOW CONDUCTANCE (2pS) KAINATE CHANNELS THAN HIGH CONDUCTANCE (20pS) ONES. L. M. Nowak and J. L. Christiansen*, Dept. of Pharmacology, NYSCVM at Cornell University, Ithaca, N.Y. 14853.

Ascher and Nowak (1988; *J.Physiol.*399: 227) previously reported that kainate application (50-100μM) activates two populations of ion channels in outside-out patches from mammalian neurons, one with an estimated conductance of 2pS and open time of 3-4ms, and the other with an estimated conductance of 20pS channel and open time of <1ms. The conductances could be observed separately in different patches and it was suggested they may to be associated with different kainate receptors; however, both responses were observed together in most patches.

Recently, we studied kainate-receptor channels and determined that the 2pS channel is activated by lower doses of kainate (5μM) than the 20pS channel (>40 μM) in the same patches at –60mV. Recordings were performed on outside-out patches from cultured mouse brain neurons. The bath solution contained 300 nM TTX and (in mM): 150 NaCl, 2.8 KCl, 1 $CaCl_2$, 10 Hepes-Na (pH 7.2). The pipette solution contained (in mM): 140 CsCl, 10 Hepes-K (pH 7.2) and 10 EGTA/1Ca. Kainate (5-100μM) was applied in the bath solution by direct superfusion from a Pasteur pipet. 5-20 μM kainate evoked relatively large inward currents accompanied by a small increase in noise. Analysis of the variance in the noise indicated activation of a 2-3pS channel. Power spectral density analysis suggested the channel open time (τ) was near 10ms. Application of 80-100μM kainate to these patches gave an estimated channel conductance of 5-6pS from variance analysis and power spectra fitted by two Lorenzians, one a very high frequency component (<1ms), and the other one about 3-4ms. Thus, the shift in the aggregate conductance from about 2pS to 5-6pS suggested that increasing the kainate dose recruited the higher conductance (20pS) channels. This idea was supported by changes in the power spectra, which began to show a second high frequency component that contributed proportionally more to the total power as kainate concentration was increased. In the recruitment, the lower frequency process (τ = 10ms) was obscured and estimated open time approached 3-4ms in >50μM kainate.

420.17

GLUTAMATE-ACTIVATED CHANNELS IN DISSOCIATED ADULT SPINAL CORD CELLS. D.O. Smith, J.L. Rosenheimer, F. Zufall*, C. Franke*, and H. Hatt*. Dept. of Physiol., Univ. of Wisconsin, Madison, WI 53706 and Physiol. Inst., Tech. Univ., Munich, Germany.

Glutamate depolarizes motoneurons in adult spinal cord. It is unclear, however, whether glutamate-sensitive channel characteristics seen in cultured embryonic cells are also present in adult animals. Therefore, membrane currents in response to pressure application of glutamate agonists were examined in acutely dissociated spinal cord cells from adult rats using outside-out and whole-cell recording techniques. 400-μm slices were cut from rat lumbar spinal cords, and 1-mm punches of gray matter were removed from the ventral horns. Following incubation in trypsin, cells were dissociated mechanically and plated for electrophysiologic analysis. Action potentials could be induced by depolarization in cells with resting potentials of approximately -60 mV. Desensitizing 27-pS NMDA-sensitive channels were recorded; conductance was voltage-dependent, and addition of 1-μM glycine increased whole-cell currents. Likewise, desensitizing 10-pS quisqualate-sensitive channels and nondesensitizing 12-pS kainate-sensitive channels were observed. Supported by NIH grant NS13600 and SFB 220.

ION CHANNELS: LIGAND-GATED II

421.1

RECEPTOR FAMILIES: cDNAs IDENTIFIED BY PCR & DNA HOMOLOGIES. **D.M. Donovan, G.R. Cutting*, B.F. O'Hara, S.S. Shimada and G.R. Uhl. Lab. of Mol. Neurobiol., NIDA/ARC, Depts. of Neurol. & Nsci., and Center for Med. Gen. and Dept. of Pediatrics, Johns Hopkins Sch. of Med., Bx 5180, Baltimore, MD 21224.**

We have used polymerase chain reactions (PCR) and other DNA homology strategies to identify cDNAs encoding receptors. PCR using oligonucleotides directed against conserved regions of the GABA-A and glycine receptors has yielded cDNAs which are similar to known ligand gated ion channels. One PCR product and the corresponding cDNA from a human retina library displays homologies to known receptors that range from 30-36% for the GABA-A subunits to 30% for the glycine subunits. Preliminary Northern blot analysis of rat mRNA and data obtained by screening libraries from human tissues suggest that mRNAs encoding this receptor are present at low frequency in the brain and higher frequency in the retina. Southern analysis and genomic cloning suggest the presence of two closely related genes in human, monkey, mouse and rat.

In a second search, a rat library was screened with an oligonucleotide homologous to the "RDC7" clone originally isolated with PCR technology by Vassart and collaborators. Preliminary DNA sequence analysis of one rat brain cortex cDNA isolate has revealed a 70% DNA sequence homology with the RDC7 from dog. Further characterization will be necessary to assign a functional role of these apparent novel receptor-like clones.

421.2

A HIGH AFFINITY RECEPTOR FOR INOSITOL-1,4,5-TRISPHOSPHATE PURIFIED FROM BOVINE CEREBELLUM AND RECONSTITUTED INTO PLANAR LIPID BILAYERS FORMS A LIGAND-ACTIVATED Ca^{2+}-PERMEABLE CHANNEL

S.R. Hingorani#, K. Ondrias*, W.S. Agnew# & B.E. Ehrlich*
#Dept. of Cellular and Molecular Physiology, Yale Univ. Sch. Med., New Haven, CT 06510 and *Depts. of Medicine and Physiology, Univ. of Connecticut, Farmington, CT 06032.

Binding studies performed with detergent extracts of rat and bovine cerebellar membranes suggested the presence of two classes of binding sites for inositol-1,4,5-trisphosphate (IP_3). One class bound [^{3}H]-IP_3 with low affinity (K_d ~ 120 nM), and was not blocked by high concentrations of free Ca^{2+}, while a second bound ligand with high affinity (K_d = 5 nM), and was completely inhibited by Ca^{2+} (IC_{50} = 300 nM). Taking advantage of a novel affinity purification procedure, the high affinity receptor was purified to near homogeneity; this protein exhibited a K_d of 4 nM, was not inhibited by free Ca^{2+}, and was formed of a single peptide of M_r = 243,000. The purified preparation was reconstituted into planar lipid bilayers and demonstrated to form an IP_3-activated, Ca^{2+}-permeable channel having a unitary conductance of 10 pS. The channel tended to open in bursts, with a low overall opening probability of 5-10%. Activity was blocked by low concentrations of heparin (10 μg/ml), but appeared to be insensitive to ruthenium red. Many records showed concerted openings and closings of integral multiples of the unitary conductance, strongly suggesting that gating may be cooperative. As these receptors are present at high densities in intracellular membranes of Purkinje cells, such interactions may be physiologically significant.

421.3

ALCOHOL MODULATION OF DESENSITIZATION OF GABA RECEPTOR-Cl^- CHANNEL COMPLEX IN RAT DORSAL ROOT GANGLION NEURONS. M. Nakahiro, O. Arakawa and T. Narahashi. Dept. of Pharmacol., Northwestern Univ. Med. Sch., Chicago, IL 60611.

We have previously reported that n-alcohols enhance GABA-induced current before desensitization occurs (Nakahiro et al., FASEB J. 4, A1201, 1990). Ethanol and n-octanol have now been found to modulate the desensitization process of GABA receptor-Cl^- channel complex. The whole-cell patch-clamp technique was used to record currents from the rat dorsal root ganglion neurons in primary culture. Bath application of 0.3 mM GABA generated a peak current that decayed to a steady-state level. The initial phase of current decay was fit by a single exponential function, and the time constant was measured. The time constant of recovery from desensitization was also determined by paired applications of 0.3 mM GABA at various intervals. Ethanol (0.3-1 M) and n-octanol (30-100 μM) decreased the time constants of both phases in a dose-dependent manner. n-Octanol (100 μM) suppressed, rather than enhanced, the current after it had been desensitized to a steady-state level. However, ethanol (0.3 M) caused no significant suppression of the desensitized current. The results indicate that alcohols modulate GABAergic neurotransmission in a phasic and tonic manner. Supported by ADAMHA grant AA07836.

421.4

ALCOHOLS INDUCE Cl^- CURRENT IN RAT DORSAL ROOT GANGLION NEURONS. O. Arakawa, M. Nakahiro and T. Narahashi. Dept. of Pharmacol., Northwestern Univ. Med. Sch., Chicago, IL 60611.

We have already demonstrated that alcohols exert an enhancing effect on GABA-activated Cl^- current, which may contribute to their depressant action on the CNS. We now report that alcohols themselves induce Cl^- currents in the rat dorsal root ganglion neurons in primary culture. Whole-cell patch-clamp technique was used. Bath application of n-octanol (0.3-3 mM) generated an inward current at -60 mV when both external and internal solutions contained 142 mM Cl^-. The current attained a peak and decayed to a lower steady-state level in a manner dependent on the concentration. n-Hexanol (3-10 mM), n-butanol (100-300 mM) and ethanol (1-3 M) also generated similar inward currents in a concentration-dependent manner. When the internal Cl^- was partially replaced by glutamate, the reversal potential for the peak current induced by octanol was shifted as expected from the Nernst equation for Cl^-. Bicuculline (10 μM) and picrotoxin (30 μM) suppressed the octanol-induced current reversibly, whereas chlordiazepoxide (10 μM) and pentobarbital (10 μM) enhanced it. These results suggest that alcohols interact specifically with a certain receptor site(s) in the GABA receptor-Cl^- channel complex thereby opening the channel. Supported by ADAMHA grant AA07836.

421.5

MODULATION OF TRANSMITTER-ACTIVATED MEMBRANE CURRENTS IN CULTURED SPINAL CORD NEURONS BY VARIOUS ADAMANTANE DERIVATIVES. H. Lampe*, H. Bigalke*, (SPON: Europ. Neurosci. Ass.), Department of Pharmacology and Toxicology, Med. School of Hannover, 3000 Hannover 61, FRG.

The agents 1-aminoadamantane and 1-amino-3,5-dimethyladamantane have been used in the treatment of M. Parkinson and movement disorders. In contrast to 1-aminoadamantane, 1-amino-3,5-dimethyladamantane and two other adamantane derivatives, 1-amino-3-isopropyladamantane and 1-amino-3,5-diethyladamantane suppressed picrotoxin-induced hyperactivity in cultured neurons, which indicates also an anticonvulsant potency (Netzer, R. and Bigalke, H., Europ. J. Pharmacol., in press). To elucidate the underlying mechanisms, effects of the drugs on chemosensitive membrane currents were investigated. We used the whole-cell patch clamp technique to record glycine- and glutamate-activated whole-cell currents. Cultures were continuously superfused with growth medium containing 4 mM Mg^{2+}. Patch pipettes were filled with low calcium/high potassium saline. Either glycine or glutamate were applied by pressure ejection to cells clamped at -60 mV membrane potential. Each of the drugs reduced the glutamate-activated current in a concentration-dependent fashion, except for 1-aminoadamantane which had no effect. The glycine-activated current was promoted by 1-amino-3,5-dimethyladamantane at concentrations of between 0.1 μM and 5 μM, but was reduced at higher concentrations. 1-Amino-3,5-diethyl- and 1-amino-3-isopropyl-adamantane promoted this current over the whole concentration range. 1-Aminoadamantane, however, did not modulate this current either. Our results support the hypothesis that an antagonistic action at the glutamate receptor or an agonistic effect on the glycine receptor may contribute to the anticonvulsant potency of the former drugs. Their effectiveness may be due to the fact that they are more lipophilic than 1-aminoadamantane.

421.6

EFFECTS OF DELTA-9-TETRAHYDROCANNABINOL ON WHOLE-CELL ION CURRENTS IN CULTURED RAT HIPPOCAMPAL NEURONS. Robert E. Hampson, Shao Wang*, Barbara A. Bennett, Mariana Morris and Sam A. Deadwyler, Department of Physiology and Pharmacology, Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, North Carolina 27103.

Studies in this laboratory have demonstrated an effect of delta-9-tetrahydrocannabinol (THC), the psychoactive ingredient of marijuana, on hippocampal neural activity (Heyser *et al.*, SN Abstr., **15**:1170, 1989; Campbell *et al.*, JPET, **239**:941-945, 1986) in awake, freely-moving rats. A recent report by Herkenham *et al.* (PNAS, **87**:1932-1936, 1990) demonstrates binding of a radio-labelled THC agonist (CP-55,940 -- Pfizer) to a high-affinity THC receptor in rat hippocampus. To examine THC-receptor interactions, we applied whole-cell patch clamp techniques to primary cultures of dissociated hippocampal neurons.

Voltage-clamp recordings from cells in culture for 5-10 days, using KCl-filled patch-clamp pipettes showed an inward membrane current at resting potential following pressure pipette application of 1 mM tp 30 mM THC to the outside of hippocampal neurons. The inward current was increased at hyperpolarizing potentials and reversed at depolarizing membrane potentials. Reversal of this current was consistent with the equilibrium potential for chloride using high chloride concentrations in the patch pipette, and was mimicked by application of of GABA in concentrations similar to those of THC. Substitution of acetate for chloride in the recording pipette produced only outward currents. Substitution of chloride-free bathing medium reduced or eliminated the inward current.

Bath application of THC also reduced voltage-dependent sodium and potassium currents. Comparison of THC with other THC analogs for ligand-gated membrane currents will be described.

[Supported by grants DA00119, DA03502 & DA04441 to S.A.D., DA05073 to B.A.B. and NS22492 to M.M.]

421.7

DIVALENT METAL CATIONS ACT AT TWO SITES TO INHIBIT 5-HT_3 RECEPTOR-MEDIATED ION CURRENT
David M. Lovinger, Section of Electrophysiology, Lab. of Physiol. Pharmacol. Studies, NIAAA, Rockville, MD 20852.

Recent studies indicate that divalent metal cations such as Zn^{2+} and Cd^{2+} interact with $GABA_A$ and glutamate receptor/channel complexes at micromolar concentrations. The effect of metal ions on the 5-HT_3 receptor/channel complex was examined in whole-cell patch-clamp recordings from NCB-20 neuroblastoma cells. Micromolar concentrations of Cd^{2+}, Cu^{2+} and Zn^{2+} inhibited ion current activated by application of 5-HT to NCB-20 cells voltage-clamped at negative membrane potentials with the order of potency being Zn^{2+} (IC_{50}=20 μM)$\geq Cu^{2+}$ (IC_{50}=25 μM)$>Cd^{2+}$ (IC_{50}=75 μM) at -50 mV. Other cations tested (Ba^{2+}, Co^{2+}, Mg^{2+}, Mn^{2+} and Ni^{2+}) were without effect at concentrations up to 200 μM. Inhibition by Cd^{2+} and Zn^{2+} was voltage-dependent (apparent K_d for inhibition increased e-fold per 72 mV and 52 mV respectively) suggesting that these ions act at a site within the channel. This contrasts with the voltage-independent action of these ions on glutamate- and GABA-gated ion channels. Inhibition by Cu^{2+} exhibited little voltage dependence with the K_d changing e-fold per 233 mV. Inhibition by each of the ions decreased with increasing agonist concentration. For example, Cu^{2+} produced 68$\pm$4% (mean$\pm$SEM) inhibition at 1 μM 5-HT, 53$\pm$3% inhibition at 2 μM, 11$\pm$1% inhibition at 5 μM and 4$\pm$3% inhibition at 10 μM. These data suggest a model in which higher concentrations of agonist increase the probability of the receptor complex assuming a configuration which is insensitive to these ions. In sum, Cd^{2+}, Cu^{2+} and Zn^{2+} potently inhibit 5-HT_3 receptor-mediated ion current via mechanisms different from their actions at other receptor/channel complexes.

421.8

SEROTONIN REDUCES AN INWARDLY RECTIFYING CESIUM SENSITIVE CURRENT IN CEREBELLAR PURKINJE CELLS (PCs) UNDER VOLTAGE CLAMP. Y. Wang*, S.-J. Li,*, J.C. Strahlendorf, and H.K. Strahlendorf., Physiol. and Neurol., Texas Tech Univ. Hlth. Sci. Ctr., Lubbock, TX 79430.

We have previously reported excitatory and inhibitory effects of serotonin (5-HT) on cerebellar PCs. We hypothesize that these multiple actions may arise in part from coupling of various 5-HT receptors to different ion channels present in PCs, including the inward rectifying cationic current (I_h) originally described in these cells by Crepel (1986). Slices from rat cerebellum were superfused with a standard Kreb's solution. PCs were voltage clamped with a single electrode switch clamp amplifier. Ten mV hyperpolarizing steps from a holding potential of -60 to -65 mV to command potentials of -120 mV elicited time - and voltage - dependent inward current relaxations with amplitudes up to 3 nA. Bath applied CsCl, 10 mM, readily blocked these inward relaxations. Superfused 5-HT, 100 μM, markedly attenuated I_h in the the majority of cells and in some cases caused an outward shift of the holding current. These effects were reversible. The 5-HT selective agonist, 8-OH-DPAT, mimicked the actions of 5-HT. We conclude that 5-HT inhibits PCs in part by reducing I_h, possibly through 5-HT_{1A} receptor mediation. Supported by NS 19296 and the Tx. Adv. Res. Prog., Grant 010674-020.

421.9

ENDOTHELIN-1 PRODUCES MEMBRANE DEPOLARIZATION ACTIVATING CALCIUM AND CHLORIDE CURRENTS THROUGH CYCLIC GMP DEPENDENT SYSTEM. T. Nishimura*1&2, T. Akasu*1, and J. Krier[2] Dept. of Physiol. Kurume U.[1] 67 Asahi-machi Kurume 830 Japan and Dept. of Physiol. Michigan State U.[2] E. Lansing, MI 48824

The ionic mechanism(s) underlying endothelin-1 (ET)-induced membrane depolarization of neurons was studied. Membrane currents in rabbit vesical pelvic ganglia were recorded in vitro using single electrode voltage clamp. Cells were impaled by electrodes filled with 2 M CsCl in a modified Krebs solution (36°C) containing 50 mM TEA, 2 mM CsCl and 300 nM TTX. At a holding potential of -60 mV ET (0.1-2 μM) induced an inward current (Iin; 0.2-2.6 nA) followed by an outward current (Iout; 0.1-1 nA). Iin and Iout were associated with increased and decreased membrane conductance respectively. In Ca-free solution Iin was reduced to 28±3% (n=6) of control, while Iout was blocked. Ca-insensitive Iin was blocked by 4-acetamido-4'-isothiocyanostilbene-2,2-disulphonic acid (0.5 mM; chloride channel blocker). Reversal potential of Iin, estimated by current-voltage relationship, were +42±3 mV (n=4) and -18±4 mV in normal Ca^{2+} (2.5 mM) and Ca-free solution, respectively. Effects of ET were mimicked by application of dibutyryl guanosine 3':5' cyclic monophosphate (db-cyclic GMP; 10-200 μM). Pretreatment of db-cyclic GMP (10 μM for 10 min) blocked endothelin-induced current responses. We conclude that ET activates both Ca^{2+} and Cl^- conductance through a cyclic GMP-dependent signal transduction system.

421.10

ON THE POTASSIUM CONDUCTANCE INCREASE ACTIVATED BY DOPAMINE IN THE JELLYFISH, *Polyorchis penicillatus*. **J. M. Chung and A. N. Spencer. Dept. of Zoology, Univ. of Alberta, Edmonton, Alta, Canada, T6G 2E9.**

The effects of dopamine (DA) applied by picospritzing onto cultured swimming motor neurons of *Polyorchis penicillatus* were examined using whole-cell recording technique. DA caused hyperpolarization and a decrease in the firing frequency. The hyperpolarizing effect of DA was accompanied by a fall in input resistance. Under voltage clamp, DA produced outward currents associated with a conductance increase and showed desensitization with prolonged agonist applications. The DA effects were concentration dependent (effective range 10nM to 100μM). The DA currents reversed polarity around -55mV close to the potassium equilibrium potential. Altering the K ion gradient across the membrane shifted the reversal potential of the response in a Nernstian manner. Taken together, the result strongly suggests the participation of K ions. The possibility of an increase in the Cl ion conductance is doubtful, because there are no shifts of the reversal potential as the Cl ion gradient across the cell changes. ***(NSERC grant A0419 to ANS.)***

PEPTIDES: PHYSIOLOGICAL EFFECTS III

422.1

EFFECTS OF A NEW TRH ANALOGUE, YM-14673 ON NEUROLOGICAL DEFICITS IN RATS SUBJECTED TO BOTH INTERNAL CAPSULE LESION AND MOTOR EXERCISE. M.Sasamata*, S.Kawasaki*, S.Yatsugi*, A.Iwai*, S.Kawabata, F.Wanibuchi, and M.Yamamoto*. Central Res. Labs., Yamanouchi Pharmaceutical Co. LTD., Tsukuba, Ibaraki 305, Japan.

Effects of YM-14673, a potent and long lasting new TRH analogue were studied in rats subjected to electrical destruction of the left internal capsule(IC) under the motor exercised condition.

In this model, neurological deficits such as hemiplegia, and decrease in amplitude of EMG activity evoked by electrical stimulation of the left sensory motor cortex were observed on the right legs. Both motor exercise using swimming, tread-mill or wheeling drum techniques, and drug administration started from 4 days after surgical operation and repeated once a day for 10-16 days. Motor exercise using wheeling drum technique accelerated the recovery of neurological deficits in IC rats. Furthermore, YM-14673 (0.03 mg/kg i.p.) accelerated the recovery of neurological deficits in IC rats both with and without motor exercise. These results suggest that the beneficial pharmacological properties of YM-14673 encourage the therapy in stroke patients with rehabilitation.

422.2

STEADY-STATE FEEDING INDUCED IN RATS BY PUSH-PULL PERFUSION OF NEUROPEPTIDE-Y (NPY) IN MEDIAL HYPOTHALAMUS: DOSE DEPENDENCE AND FOOD PALATABILITY. X. Paez* and R. D. Myers. Department of Pharmacol., School of Medicine, East Carolina University, Greenville, NC 27858.

When perfused in medial hypothalamic sites of unrestrained rats, NPY produces an apparent not-satiable feeding behavior. In these experiments, this region was perfused repeatedly with an artificial CSF, for 6 min intervals, by means of push-pull cannulae in rats given *ad lib* pelleted rat chow and chocolate biscuits. Two perfusions of 0.2 μg/min NPY induced marginal feeding only at the control level, (i.e., <2.0 g in 2.0 hr) but at a dose of 2.0 μg/min, the rat consumed 7.8±1.9 g of food over 2.0 hrs. A sequence of 10 perfusions of the lower dose NPY induced an intake of 14.2±2.0 g over 5.0 hrs, whereas the higher dose evoked an intake of 31.7±3.3 g over the same time interval. When rats were offered only chow during 10 perfusions of 2.0 μg/min NPY, they ate less (i.e., 5.0 - 13.0 g in 4.0 hr) than when chocolate biscuits were simultaneously available (i.e., 26.0-28.0 gm in 4.0 hr). Intakes of water during perfusion of the higher dose did not increase irrespective of the amount of food consumed by the rat. Thus, palatability of food interacts with the level of NPY in hypothalamic tissue to determine the magnitude of food intake induced by the peptide. Supported by NSF Grant 84-10063 to RDM.

422.3

GALANIN AND TRH EXCITE SPINAL DORSAL HORN NEURONS **K. Mirnics, Z. Korade and S. Jeftinija. Dept. Vet Anatomy, Iowa State Univ. Ames, IA 50011, USA.**

Galanin and thyrotropin-releasing hormone (TRH) were shown to be present in superficial laminae of the dorsal horn (DH), but the data regarding their physiological function are not available. In order to investigate the effect of these two peptides we employed a technique of intracellular recording from DH neurons in an in vitro horizontal spinal cord slice preparation. The preparation consisting of a rat spinal cord DH slice (300-500 μm thick) with intact dorsal roots (DR). Intracellular recordings from single DH neuron was performed with 3M K-acetate back-filled glass microelectrodes having DC resistance of 100-150 MΩ. Dorsal roots were stimulated by using bipolar platinum stimulation electrodes. The thresholds for activating large myelinated and small unmyelinated afferents have been determined by recording action potentials in DRG neurons and have been found to be 8-20V/0.02ms for the most sensitive axons and over 35V/0.5ms for C-fibers. Bath application of galanine (10^{-12}M to 10^{-6}M) produced a dose dependent depolarization in about half of the neurons tested (n=15). The depolarization was associated with an increase in membrane conductance and a very prolonged firing of action potential in some neurons (n= 5). In addition to increase in spontaneous firing, galanine had an facilitatory effect on synaptic activation evoked by dorsal root stimulation at intensities sufficient to activate myelinated and unmyelinated afferents. TRH produced depolarization was associated with increase in conductance and firing of action potentials. Work was supported by NIH grant NS27751 and USDA grant PL95-113.

422.4

MORPHINE MODULATES THE INDUCED RELEASE OF FMRF-NH-$_2$-LIKE PEPTIDE FROM RAT SPINAL CORD. J.M. Zhu and H.-Y.T. Yang Lab. of Biochem. Genetics, NIMH, Neuroscience Center at St. Elizabeths, Washington, DC 20032.

Several lines of evidence indicate that FMRF-NH_2-like peptide, FLFQPQRF-NH_2 (F-8-F-NH_2) isolated from bovine brain, may have a role in opioid mediated antinociception. In rat spinal cords, high density of F- 8-F-NH_2 immunoreactive terminals was detected in superficial laminae of dorsal horn and the presence of specific F-8-F-NH_2 receptors was also demonstrated. These studies suggest that F-8-F-NH_2 may function as neurotransmitter or neuromodulator in the spinal cords and effect of morphine on this secretion were studied by an in vitro superfusion procedure. The spontaneous release of F-8-F-NH_2 was high at the begining of the superfusion and then gradually decreased to a steady level of 7 fmol/ml in a calcium dependent manner when KCl in the perfusion medium was increased to 56 mM. The released F-8-F-NH_2 immunoreactivity was analyzed by HPLC followed with RIA and the main immunoreactivity released was found to be identical to the main immunoreactivity detected in the rat spinal cord extract. Addition of morphine to the perfusion medium was found to inhibit the 56 mM KCl induced release of F-8-F-NH_2 in a dose dependent manner. These results are in good agreement with the hypothesis that F-8-F-NH_2 may participate in opioid mediated antinociception.

422.5

SUBTYPES OF GUANINE NUCLEOTIDE-BINDING REGULATORY PROTEINS INVOLVED IN THE SUPPRESSION OF BARORECEPTOR REFLEX BY NEUROTENSIN IN THE RAT. Samuel H.H. Chan, M.J. Fu*, K.S. Lin* and Julie Y.H. Chan. National Yang-Ming Med. Coll., and Taipei Veterans General Hospital, Taipei, Taiwan, ROC.

We attempted to identify the subtypes of guanine nucleotide-binding regulatory proteins (G-proteins) that may underlie the suppressive effect of neurotensin (NT) on the baroreceptor reflex (BRR), using Sprague-Dawley rats that were anesthetized with pentobarbital sodium. Intracerebroventricular (i.c.v.) application of NT (15 nmol) significantly inhibited the BRR response. Such an inhibition was appreciably antagonized by pretreating animals with i.c.v. injection of pertussis toxin (10 or 20 pmol), which ADP-ribosylates Gi, Gp and Go; n-ethylmaleimide (1 or 2 nmol), which uncouples Gi from GTP by alkylation; forskolin (30 or 60 nmol), which activates adenylate cyclase; or phorbol 12-myristate-13-acetate (2 or 4 nmol), which stimulates protein kinase C; but not by cholera toxin (15 or 30 nmol), which ADP-ribosylates Gs. More specifically, the effects of these pretreatments on NT-induced suppression of BRR were essentially duplicated upon bilateral microinjection into the nucleus tractus solitarius of pertussis toxin (80 or 160 fmol), n-ethylmaleimide (16 or 80 pmol), forskolin (240 or 480 pmol), or phorbol ester (16 or 32 pmol); but not by cholera toxin (120 or 240 fmol). These results suggest that a pertussis toxin-sensitive G-protein(s), possibly Gi or Gp, may be involved in the suppression of BRR response by NT at the nucleus tractus solitarius.

422.6

EXCITATORY ACTION OF SOMATOSTATIN (SST) ON RAT AMBIGUAL MOTONEURONS *IN VITRO*. Y.T. Wang, R.S. Neuman & D. Bieger. Faculty of Medicine, Memorial University of Newfoundland, St. John's, Newfoundland, Canada, A1B 3V6

The occurrence of SST in subnucleus centralis (NTS_c) neurons of the solitary complex has recently been reported. NTS_c neurons send a dense projection to the compact oesophagomotor subdivision of the ambiguus complex (AMB_c). Using intracellular recording with KCl (3M) or K-methyl-SO_4 (2M) filled microelectrodes from sagittal brainstem slices perfused at 32°C in a submersion chamber, we have investigated the effects of SST on AMB_c neurons. Pneumophoretic application of SST induced a) an immediate dose-dependent membrane depolarization with associated spiking, b) little or no change in membrane conductance, and c) an inward current recorded under voltage-clamp in the presence of TTX. There was no change in post-spike hyperpolarization and no evidence of desensitization with test intervals of 2 min.

We conclude that SST is a prime candidate for excitatory transmitter in the NTS_c-AMB_c pathway and that its role in oesophageal peristalsis merits further scrutiny.

Supported by MRC (Canada)

422.7

RESPONSE OF NEURONS IN THE DORSAL MOTOR NUCLEUS OF THE VAGUS TO THYROTROPIN-RELEASING HORMONE (TRH). C.A. Livingston and A.J. Berger. Dept. of Physiology & Biophysics, Univ. of Washington School of Medicine, Seattle, WA 98195.

Preganglionic parasympathetic neurons of the dorsal motor nucleus of the vagus (DMX) regulate visceromotor activity. Using an *in vitro* slice preparation of the guinea pig brainstem, we determined the effects of TRH (one of numerous neuropeptides localized within the nucleus) on DMX neurons. Slices (350 μm thick) from caudal medulla were superfused with standard Ringer's solution, and the responses of DMX neurons to bath-applied TRH were recorded in current-clamp conditions. In all cells tested, TRH (5-10 μM) induced a reversible 2-18 mV (average, 9 mV) depolarization, typically accompanied by a decrease in membrane conductance, which persisted over many minutes. A similar response was evoked in the presence of low Ca^{2+}, high Mg^{2+} (Ca^{2+} spikes and Ca^{2+}-dependent K^+-currents abolished), indicating that TRH acts directly on these cells. TRH applied in the presence of 0.5 μM TTX or 0.5 μM TTX and 2 mM CsCl similarly depolarized these cells, suggesting that the response is not mediated exclusively via a TTX- or Cs^+-sensitive current. Evaluation of the I-V relations of the cells in the presence and absence of TRH indicates the reversal potential of the response to be -75 to -90 mV. We suggest that the TRH-induced depolarization of DMX neurons is due to a reduction of an outward cationic current. (Supported by NS 14857 and a Parker B. Francis Foundation Fellowship)

422.8

ANGIOTENSIN II AND SUBSTANCE P EXCITE NEURONS IN THE RAT MEDIAL NUCLEUS TRACTUS SOLITARII (mnTS) IN VITRO. K. L. Barnes, W. D. Knowles and C. M. Ferrario. Departments of Brain and Vascular Research and Neurology, Research Institute, Cleveland Clinic Foundation, Cleveland, OH 44195.

There is a striking congruence between the patterns of high affinity binding sites and neuronal elements immunoreactive for angiotensin II (Ang II) and substance P (SP) in the rat mnTS. Although both peptides influence autonomic function via this region, Ang II is thought to subserve cardiovascular function, while SP appears to exert broader sensory and autonomic effects. To examine whether these peptides act on the same or different neurons, we compared the effects of Ang II and SP on mnTS neurons recorded from in vitro slices of the rat medulla. Horizontal slices (400 μm) containing the mnTS were perfused with artificial cerebrospinal fluid (aCSF, 36°C). Extracellular recordings were obtained from 20 mnTS neurons during microdrop application of Ang II or SP (both 1 μM in aCSF) or aCSF onto the slice surface. Ang II substantially increased the firing rate of 8 of the 20 cells tested, while SP excited 10 of 14 cells given this peptide. The time course of the response to SP was similar to that for Ang II. No inhibitory effects were seen with either peptide. In 14 neurons the effects of Ang II and SP were compared. Three cells responded only to Ang II, 6 neurons were excited only by SP, 4 cells were excited by both peptides, and 1 neuron did not respond to either peptide. These studies reveal a partial overlap of the neuronal substrate that mediates the autonomic effects of Ang II and SP via the mnTS, and suggest that there may be interactions between Ang II and SP in certain pathways of the mnTS. (Supported in part by NHLBI grant HL-6835).

422.9

ANGIOTENSIN II INHIBITS GLUTAMATE-EVOKED DEPOLARIZATION OF LOCUS COERULEUS NEURONS IN VITRO

Huangui XIONG and Kenneth C. MARSHALL Dept. of Physiology, University of Ottawa, 451 Smyth Road, CANADA K1H 8M5

The effects of iontophoretically applied angiotensin II (AII) on intracellularly recorded locus coeruleus (LC) neurons were studied in transverse pontine slices from rats weighing 50-100 gm. The major effect of AII was a specific depression of the depolarization and resultant excitation produced by iontophoretic glutamate (Glu) application (39/47 neurons). Application of Na^+ ions with equal or higher currents had no effect on membrane potential or responses to glutamate. The action of AII was accompanied by a small hyperpolarization of the cell membrane potential in a few neurons. However, in most cells, the depression of Glu actions occurred in the absence of changes in membrane potential, or amplitude, duration or shape of the action potential. AII had no effect on excitation evoked by injection of depolarizing current, or by application of acetylcholine and thus appears to specifically inhibit Glu actions. The effects of AII on Glu excitations were antagonized by [Sar^1, Val^5, Ala^8]-AII in 9/12 neurons. The AII effects were not changed by superfusion with low Ca^{++}/high Mg^{++} (0.5 mM/10 mM) solution, indicating a postsynaptic site of action. These observations indicate that AII can exert a selective and potent inhibitory modulation of the postsynaptic actions of glutamate.

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422.10

NEUROPEPTIDE Y (NPY) INHIBITS α_1-ADRENERGIC AND 5-HT_{1A} MEDIATED SYNAPTIC POTENTIALS IN DORSAL RAPHE *IN VITRO*. Samuel B. Kombian and William F. Colmers. Dept of Pharmacology, Univ. of Alberta, Edmonton, Alberta, CANADA T6G 2H7.

Because the (entirely extrinsic) noradrenergic innervation of the largely serotonergic dorsal raphe nucleus also expresses NPY, receptors for this peptide are found there, and NPY has been shown to inhibit transmitter release in several tissues, we examined the effects of NPY on synaptic potentials in dorsal raphe. Intracellular recordings were made with 2M KCl-filled microelectrodes of rat dorsal raphe nucleus neurons in submerged *in vitro* slices. Focal electrical stimuli from bipolar tungsten electrodes placed in the nucleus were used to evoke synaptic potentials. Fast excitatory amino acid (EAA) and $GABA_A$ potentials were succeeded by a slower, 5-HT_{1A} autoreceptor-mediated IPSP and an even slower, α_1 adrenoceptor-mediated EPSP.

The slow EPSP evoked by focal stimuli averaged 3.9±0.6 mV, and peaked at 1833 ± 50ms after the stimulus. This EPSP was completely eliminated by bath application of prazosin (100nM). Bath application of 1μM NPY caused a decrease in amplitude of the slow EPSP by 45.3 ± 9.6% (n=7, $P < 0.05$), which reversed fully upon washout of the peptide. The slow, 5-HT_{1A} autoreceptor-mediated IPSP was decreased in amplitude by NPY from 12.9 ± 1.1 mV to 10.6 ± 1.1 mV, a decrease of 18.6 ± 2.4% (n=7, $P < 0.001$). There was no significant change in the amplitudes of the fast synaptic potentials. NPY did not alter the resting membrane potential or input resistance of these neurons.

The results indicate that NPY may act to decrease the electrically-evoked release of noradrenaline from terminals in the dorsal raphe nucleus. Moreover, NPY appears to have a slight inhibitory effect on release of 5-HT from neurons within the dorsal raphe. The site and mechanism of NPY's actions in the dorsal raphe nucleus remain to be elucidated.

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422.11

PRESYNAPTIC INHIBITION BY NEUROPEPTIDE Y (NPY) AND ADENOSINE IN RAT HIPPOCAMPUS IS BY A DIFFERENT MECHANISM THAN BACLOFEN. William F. Colmers and Gloria J. Klapstein. Dept. of Pharmacology, Univ. of Alberta, Edmonton, Alberta, CANADA T6G 2H7.

NPY presynaptically inhibits excitatory transmission between stratum radiatum and CA1 pyramidal cells. This can be prevented with 4-aminopyridine (4-AP); however, reducing extracellular calcium restores NPY's inhibition, suggesting that it inhibits some, but not all, calcium channels in presynaptic terminals (Colmers, et al, J. Neurosci 8:3827 [1988]). $GABA_B$ and adenosine A_1 receptors also mediate presynaptic inhibition at this synapse. We therefore examined whether these two receptors acted via a mechanism similar to that postulated for NPY.

Population spikes (PS), evoked by orthodromic electrical stimulation of stratum radiatum (70-90% of maximum response) were recorded with extracellular microelectrodes in the CA1 pyramidal cell layer of submerged transverse slices of rat hippocampus. Bath application of 1μM NPY, caused a reversible reduction of 93% in PS amplitude, which was reduced to only 26% in the presence of 30 μM 4-AP, and restored to 67% by 0.75mM extracellular Ca^{++}. Although 4-AP caused a small reduction in the effect of 10μM baclofen, from 97% to 76%, low Ca^{++}-4-AP had no significant effect. By contrast, no effect was seen with these treatments on the effect of 3 μM 2-CA. To more carefully examine this, dose-response relationships for NPY, baclofen and 2-CA were constructed in saline, in 4-AP and low Ca^{++}-4-AP. While 4-AP shifted the dose-response curve for NPY, 2-CA and baclofen rightward in a parallel manner, lowering Ca^{++} in the presence of 4-AP caused a parallel leftward shift only to NPY and 2-CA, while the 4-AP mediated rightward shift in the dose-response curve for baclofen was not shifted leftward by low Ca^{++}-4-AP.

The results indicate that in hippocampal CA1, presynaptic NPY and adenosine A_1 receptors may share similar mechanisms, while the presynaptic $GABA_B$ receptor may act via different mechanism than the others.

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422.12

CELLULAR EFFECTS OF ATRIAL NATRIURETIC PEPTIDE ON VASOPRESSIN NEURONS. K.M. Hurley and C.B. Saper. Depts. of Pharm. & Physiol. Sci. and Neurology, Univ. of Chicago, Chicago, IL 60637.

Atrial natriuretic peptide (ANP) is a potent natriuretic and diuretic hormone that also acts as a central neuromodulator. Systemically, ANP opposes virtually all of the effects of arginine vasopressin (AVP). Intraventricular ANP administration inhibits the secretion of AVP from the posterior pituitary gland. Application of ANP in femtomole doses to AVP neurons in the paraventricular nucleus increases the intervals between bursts of neuronal firing and shortens burst duration, but the mechanism of this effect is not known. We recorded intracellularly from magnocellular neurons in the supraoptic and paraventricular nuclei in 350-400 μm slices through the rat hypothalamus. Micropipettes were filled with a 4% solution of biocytin in 0.5 M KCl-0.05 M TRIS buffer. At the end of each experiment, cells were filled iontophoretically with biocytin and cell type was verified by combined FITC-immunofluorescence for AVP and Texas Red-streptavidin for biocytin. Cells had an input resistance in the range of 130-160 MΩ, membrane potential of -60 to -80 mV and action potential amplitudes of 60-80 mV. Membrane conductance was monitored by 2Hz, 30 ms current pulses of -0.2nA. ANP (0.01-10 fm) applied to the surface of the slice resulted in a decrease in membrane conductance and complex changes in membrane potential and cell firing pattern. Similar mechanisms may modulate AVP release in vivo.

422.13

LIGHT/DARK RHYTHM OF NEUROPEPTIDE Y-LIKE IMMUNOREACTIVITY IN DISCRETE HYPOTHALAMIC NUCLEI. **M. Jhanwar-Uniyal, B. Beck, C. Burlet[a] and S.F. Leibowitz. The Rockefeller Univ. New York, NY 10021 and Faculte de Medicine, INSERM U.308, Nancy (France).**

Neuropeptide Y (NPY), a putative neurotransmitter abundant in the paraventricular nucleus (PVN) of the hypothalamus, induces a robust eating response, in particular carbohydrate intake, which is strongest specifically at the onset of the active cycle. Moreover, evidence indicates that NPY modulates circadian rhythms of activity level. In this study, we investigated whether endogenous NPY, measured in 9 hypothalamic nuclei, may itself vary in relation to the light/dark cycle.

Sprague-Dawley rats were kept on a 12:12 hr light:dark cycle, with lights out at 19:00 hr. They were sacrificed at one of the following times: 1 hr before dark onset (18:00 hr); 1 hr after dark onset (20:00 hr); in the middle of dark cycle (01:00 hr); at light onset (07:00 hr); and in the middle of the light period (13:00 hr). Nine hypothalamic sites, namely, PVN (P; parvocellular), PVN (M; magnocellular), suprachiasmatic (SCN), arcuate (ARC), dorsomedial, ventromedial, supraoptic nuclei, median eminence (ME) and perifornical lateral hypothalamus, were micropunched and assayed for NPY content via RIA.

The results demonstrated that: 1) NPY content in the PVN (P), but not PVN (M), rose significantly 1 hr before the dark cycle ($F_{4,35}=2.78$; $P<0.05$), declined reliably within 1 hr after dark onset, and remained low thereafter; 2) NPY levels in the SCN peaked at two time points, at dark onset and also at light onset ($F_{4,30}$ 6.43; $P<0.001$). 3) NPY levels in the ARC exhibited a similar pattern to those detected in the SCN ($F_{4,34}=3.15$; $P<0.02$). 4) No other hypothalamic sites showed any significant temporal variation in NPY content. Based on these results and other behavioral findings, it is postulated that NPY acts physiologically in relation to the light/dark cycle, and that NPY in the parvocellular region of the PVN is involved in the control of natural feeding behavior at the onset of the dark (active) period.

422.14

THE EFFECT OF LHRH ON ^{45}Ca-UPTAKE AND IP ACCUMULATION IN RAT HIPPOCAMPUS T.L.Thompson and R.L.Moss. Dept. of Physiology, Univ. of Texas Southwestern Medical Center, Dallas, TX 75235

Luteinizing hormone releasing hormone (LHRH) receptors have been localized to discrete brain areas with the highest concentration being found in the hippocampus: the density of these receptors is very sensitive to estrogen priming. Intracellular recording data from our lab have shown that LHRH has a long acting modulatory effect on CA1 hippocampal neurons. We have initiated a series of experiments designed to elucidate which biochemical processes (second messenger systems) are affected by LHRH-receptor activation in the hippocampus; specifically examining the effect of LHRH treatment on ^{45}Ca-uptake and inositol phosphate (IP) accumulation. Hippocampal slices or minces were prepared from ovariectomized Spraque-Dawley rats. Slices were exposed to ^{45}Ca in the presence of 10^{-12}M-10^{-6}M LHRH In some experiments LHRH antagonists were added prior to the LHRH. LHRH caused a modest (15%-30%) but significant increase in ^{45}Ca-uptake. This response could be effectively blocked by Nal-Glu-LHRH (a LHRH antagonist). A 48hr priming with 5ug estradiol benzoate (EB) had no apparent effect on the absolute magnitude of the LHRH response. IP accumulation was measured in response to LHRH (10^{-6}M-10^{-10}M) in the presence of 10mM LiCl. Total IP accumulation was determined by ion exchange chromatography. LHRH caused a significant increase in IP accumulation; as much as 80% above control. This accumulation was partially inhibited by pretreatment with Nal-Glu-LHRH. EB priming resulted in a decrease in the LHRH induced increase of IP accumulation.

These preliminary findings suggest that LHRH activation of receptors in the hippocampus results in an increase in ^{45}Ca influx and IP accumulation. This is similar to that observed in the pituitary in response to LHRH. In addition, there is an indication that estrogen may modulate some of the cellular responses to LHRH in the hippocampus. Supported by grant NIH HD09988.

422.15

INSULIN INDIRECTLY STIMULATES PI TURNOVER IN RAT HIPPOCAMPUS (H). D. Figlewicz Lattemann and P. Szot.Depts. of Psychology and Medicine, Univ. Wash. and VA Med. Center, Seattle, WA 98195.

Insulin (INS) reportedly stimulates phosphorylation of two kinase-C substrate proteins in rat H. We tested whether INS stimulates PI turnover, a mechanism of kinase-C activation, in H slices. INS significantly stimulated formation of inositol phosphate (IP_1), IP_2, and diacylglycerol. 500 nM tetrodotoxin (TTX) suppressed INS-stimulated IP release, suggesting mediation by an endogenous transmitter. As INS and norepinephrine (NE) (via a-1 receptors) have similar electrophysiologic effects in rat H, we tested the a-1 antagonist prazosin (PRZ). PRZ (1 μM) stimulated IP release slightly, but reduced INS-stimulated IP release to levels similar to those with PRZ alone.

	CHANGE FROM CONTROL LEVELS, 3H-IP DPM		
INS,	0 (CONTROL) +TTX +9±17	+PRZ +141±46	
(nM)	5 +239±84	------	+93±29
	10 +204±92	-31±36	+129±47
	100 +78±30	-35±53	------

CONCLUSION: INS stimulation of PI turnover in rat H may be mediated via endogenous NE and activation of a-1 adrenergic receptors.

422.16

SEXUAL DIMORPHISM IN VASOPRESSIN-MEDIATED NEUROTRANSMISSION. D. Albeck, T. Smock, S. Arnold*, K. Raese*, K. Paynter*, and S. Colaprete. Center for Neurosciences, Department of Psychology, University of Colorado, Boulder, Colorado 80309.

A peptide similar to vasopressin or oxytocin acts as a neurotransmitter in the rat hippocampus (Brain Res., 1990, 511:7 and 15). Antisera to arginine vasopressin recognize this peptide and the pathway has been studied extensively by immunocytochemistry and other tract-tracing techniques. The peptide fibers originate in the medial amygdala and are present in greater density in males than females. The peptide is depleted completely by castration of males and is restored after testosterone replacement (DeVries et al., J. Comp. Neurol., 1985, 233:236). Here we report that peptidergic transmission, mediated by the vasopressin-like peptide, is also sexually dimorphic and eliminated by castration.

In 27 male rats, the medial amygdala was electrically stimulated to release the peptide into the hippocampus. The evoked population spike in acute field potential recording was depressed (n = 7). Individual pyramidal cells were inhibited in single unit recording (n = 17) and individual interneurons were excited (n = 3). In all of seven trials, the effect of the released peptide was blocked by a structural vasopressin/oxytocin antagonist.

Sixteen males were compared with ten females in acute field potential recording. Both females and males responded to the stimulation in the same way, but the hippocampal response in males endured longer (23 ± 6 min) than the signal in females (11 ± 6 min, $p < 0.05$). Fifteen normal males were compared with five males castrated 15 weeks previously. The peptidergic signal was not detectable in the castrates (different from normals, $p<0.02$).

The behavior of fifteen males was observed upon bilateral AME stimulation. There was a change consonant with the involvement of this system in sexual behavior.

Thus, results from physiology parallel those from anatomy that reveal sexual dimorphism and steroid dependence in this brain peptidergic system. Supported by MH45202.

422.17

OPIOID PEPTIDES REDUCE POSTSYNAPTIC POTENTIALS IN A SLICE PREPARATION OF NUCLEUS ACCUMBENS (NAcc). X. Yuan*, S. Madamba* L. Koda and G. R. Siggins, Dept. Neuropharmacology, Scripps Clinic Res. Inst., La Jolla, CA 92037.

The NAcc is of interest because of its suggested role in reward mechanisms and, possibly, opiate and cocaine abuse (Koob and Bloom, Science 242:715, 1988). This region contains abundant levels of opioid peptides, including enkephalins, dynorphins and endorphins. Therefore, we developed an in vitro slice preparation of NAcc and tested the effects of superfusion of opioid peptides on NAcc neurons. Intracellular recordings of these neurons revealed that many have unusual membrane properties, including: 1) large resting membrane potentials (avg. -81 mV; range: -68 to -94 mV; 24 neurons); 2) anomalous rectification, resulting in a 'sag' of the electrotonic potential generated by hyperpolarizing current steps; and 3) a time-dependent 'ramping' response to depolarizing current steps, resembling the effect of the D-current (Storm, Nature 336:379, 1988) of hippocampal neurons. However, other cells show a more linear I-V curve, suggesting the presence of more than one NAcc neuron type. Input resistances averaged 51 MΩ. We found little effect of superfusion of several enkephalin analogues, D-pen^2, D-pen^5 enkephalin (a δ opiate receptor agonist; n = 7), DAGO (a selective μ receptor agonist; n = 7), or D-ala^2, D-leu^5 enkephalin (a broad spectrum μ/δ agonist; n = 1) on either membrane potential or input slope resistance, at 1-2 μM. However, all of these opioids dramatically reduced postsynaptic potentials (to 41% of controls; n = 6) evoked by afferent stimulation in the area ventral to the NAcc. These results suggest that the major role of opioid peptides in this region is to reduce synaptic transmission. (Supported by NIDA grant DA 03665.)

422.18

VASOACTIVE INTESTINAL POLYPEPTIDE MODULATES ROD BIPOLAR CELL RESPONSES TO INHIBITORY RETINAL NEUROTRANSMITTERS. M.L. Veruki and H.H. Yeh, Program in Neuroscience and Dept. Neurobiology & Anatomy, Univ. Rochester Med. Ctr., 601 Elmwood Avenue, Rochester, NY 14642.

Studies from our laboratory indicate that, in the rat retina, vasoactive intestinal polypeptide(VIP)-like immunoreactivity is localized to amacrine cells. Even though ultrastructural data is as yet lacking, their processes ramify extensively in the inner plexiform layer and should thus be in a position to influence synaptic interactions in the inner retina. Here, we tested the effects of VIP on rod bipolar cells of the rat retina.

Isolated rod bipolar cells were obtained by gently triturating papain-incubated whole retinas and conventional whole-cell patch-clamp procedures were employed to examine their responses to agonists under voltage-clamp. In initial experiments, pressure application of VIP (≤100 uM) elicited no detectable currents and it was thought that the neuropeptide had no effect on rod bipolar cells. However, in subsequent experiments, the amplitude of rod bipolar cell current responses to brief (50-300 ms) pulses of either GABA (20 uM; n=7) or glycine (100 uM; n=6) were consistently augmented during concomitant exposure to 10 uM VIP. At this concentration, VIP by itself had no effect on membrane conductance. In contrast, VIP effects on GABA- and glycine-induced currents in isolated ganglion cells were relatively unpredictable, although an attentuation of responses to the inhibitory transmitters was frequently observed. It is proposed that one action of VIP may be to modulate the excitability of inner retinal neurons. With specific regard to the rod bipolar cells, such a modulation may serve to enhance the efficacy of inhibitory feedback inputs mediated by GABA or glycine.

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422.19

TRH STIMULATES INOSITOL PHOSPHATE (IP_X) RELEASE IN RAT RETINA. A. Sattin, B. Anderson*, M.J. Kubek. Depts. of Psychiatry and Anatomy, VA and Indiana Univ. Med. Centers, Indianapolis, IN 46202-4887.

Last year we reported here small increases (10-20% over baseline) in IP_X release in rat hippocampal slices using 10 nM TRH in the presence or absence of Lithium, 10 mM. Retina is a CNS structure that is also known to contain TRH and its receptors. After a 1 hr. load-up with myo-(2-^{3}H) inositol followed by a 20 min. chase with cold myo-inositol, and then a 15 min. preincubation in 10 mM Li^+, individual retinal tissues were incubated for 30 min. more without and with 10 nM TRH. Reaction was stopped by freezing the tissue in quick-transfer holders, then extracting in chloroform-methanol. ^{3}H-IP's were separated from ^{3}H-I chromatographically and total DPM's were obtained from the chloroform fraction, tissue IP's, tissue I and medium I. Results were expressed as percent of DPM's in IP's to total of DPM's of all other fractions from each sample. Results cumulate the data from three incubations. Basal IP_X in Long-Evans rats was 4.0 ± 0.9 (SEM) (N=13) vs. 5.5 ± 1.0 (N=13) with TRH (38% increase, $P < 0.0019$). Basal IP_X in Sprague-Dawley rats was 3.9 ± 0.8 (N=8) vs. 8.2 ± 1.5 (N=8) with TRH (110% increase, $P < 0.0036$). These may be the largest IP_X release effects of TRH so far reported in CNS. D-E curves and strain comparison will be pursued. Supported by VA Res. Serv.

OPIOIDS: ANATOMY AND PHYSIOLOGY I

423.1

ENDOGENOUS OPIOIDS RELEASED FROM PERFORANT PATH MODULATE NOREPINEPHRINE RELEASE AND IPSP AMPLITUDES IN GUINEA PIG CA3 PYRAMIDAL CELLS. **R.M. Caudle, J.J. Wagner*, C. Chavkin, Dept. of Pharmacology, University of Washington., Seattle, WA 98195.**

Our previous work demonstrated that pharmacologic depolarization or electrical stimulation of opioid containing pathways in hippocampal slices could release endogenous opioids to displace either [3H]-diprenorphine or [3H]-DAGO binding. In this study we demonstrate that endogenous opioids were released by perforant path (PP) stimulation to affect synaptic potentials recorded in CA3 pyramidal cells. CA3 pyramidal cells were impaled in perfused guinea pig hippocampal slices. Synaptic potentials in pyramidal cells were evoked by stimulation of the MF(150-400 μA, 0.033 Hz). A single stimulus train (150 μA, 10 Hz, 5 s) in the PP produced a small depression in IPSP conductance that lasted for approximately 15 min. The percent change when compared to unstimulated controls was -11.1 ± 8 (N=13,p<0.05). When naloxone (100 nM) was added to the bath and the PP was stimulated a large increase in conductance was observed (43.5 ± 27 % change, p<0.05, N=13). Following PP stimulation, the naloxone-induced change in IPSP conductance was delayed and prolonged: no effect was seen until 3.0 ± 0.6 min after stimulation; time to peak was 5.6 ± 0.9 min; total duration was 6.4 ± 1.0 min. The increase in IPSP conductance was specific to perforant path stimulation. Bath applied propranolol (1 μM, N=10) or pretreatment of the guinea pigs with reserpine (5 mg/kg, N=6) blocked the increase in IPSP conductance. These findings suggest that endogenously released enkephalin regulates the release of norepinephrine and that endogenously released norepinephrine enhances IPSP conductance in the CA3 region of guinea pig hippocampus. Supported by DA04123.

423.2

NALOXONE BLOCKS THE INDUCTION OF FOS-LIKE IMMUNOREACTIVITY IN THE HIPPOCAMPAL FORMATION AND CORTEX. J.L. Martinez, Jr., D.N. Lieberman, M.P. Jasper, O.I. Petukhova*, B.E. Derrick, and F.R. Sharp, Dept. of Psych., Univ. of Calif., Berkeley, CA, 94720 and Dept. of Neurol., Univ. of Calif., San Francisco, CA, 94121.

Long-term potentiation at the mossy fiber (MF)-CA3 synapse is blocked by mu opioid receptor antagonists (Lieberman et al., this volume). As a prelude to investigating the involvement of c-fos in MF LTP, we examined the effect of naloxone on the induction of fos-like immunoreactivity (FLI) in hippocampus in vivo following MF stimulation in pentobarbital anesthetized rats. Single pulse MF stimulation and/or the disruption following placement of electrodes into the CA3 region and DG induced high levels of FLI in the DG, CA fields and cortex. Five μmols of naloxone delivered unilaterally into the CA3 pyramidal layer during MF stimulation completely blocked induction of FLI in all brain areas when measured 1.5 hrs later. Activation of opioid receptors therefore may be important for expression of FLI following cellular disruption by implantation and stimulation. Since c-fos is implicated in the regulation of the proenkephalin gene in DG granule cells, naloxone inactivation of FLI induction suggests there may be a regulatory coupling between opioid receptor activation and the proenkephalin gene.

Supported by DA04195, NS24666 and the Rennie Fund.

423.3

DIFFERENT G PROTEINS MEDIATE THE OPIOID INHIBITION OR ENHANCEMENT OF EVOKED METHIONINE-ENKEPHALIN RELEASE. H. Xu and A.R. Gintzler. Department of Biochemistry, SUNY Health Science Center at Brooklyn, Brooklyn, NY 11203.

This laboratory has previously demonstrated that there is an opioid receptor mediated enhancement and inhibition of the electrically stimulated release of methionine-enkephalin (enkephalin) from the guinea pig myenteric plexus. Low doses (nanomolar) enhance, whereas higher concentrations (10-100 nM) inhibit release. We now demonstrate that the islet-activating protein from pertussis toxin (PTX; 50 ug i.p./500g for 6 days) can abolish the ability of mu, delta and kappa-selective opioids to inhibit the evoked release of enkephalin. In contrast, PTX is without effect on the enhancement of enkephalin release observed following treatment with nanomolar concentrations of the above opioids. Conversely, pretreatment with cholera toxin (CTX; 10^{-11}M for 3 hrs in vitro) has no effect on the mu, delta or kappa opioid inhibition of evoked enkephalin release but abolishes the ability of nanomolar concentrations of these agonists to enhance stimulated enkephalin release. These data further differentiate the signal transduction process responsible for each opioid effect. Furthermore, they suggest that a PTX-sensitive G protein (G_i or G_o) and a CTX-sensitive G protein (G_s) are integral components of the mechanism that mediates opioid inhibition and opioid enhancement, respectively, of evoked enkephalin release.

423.4

STIMULANTS OF PROTEIN KINASE C STIMULATE IMMUNOREACTIVE BETA-ENDORPHIN SECRETION FROM DISSOCIATED FETAL HYPOTHALAMIC CELL CULTURES. L.P. Kapcala and Horng-Heng Juang*. University of Maryland School of Medicine, Baltimore, MD 21201

Relatively little is known about the regulation of secretion of hypothalamic β-endorphin, the potent opioid which is believed to play a variety of physiological roles in brain. Previous work has shown that vasopressin, which acts in brain primarily through activation of the phosphoinositol second messenger system, stimulates secretion of hypothalamic β-endorphin. We tested the hypothesis that stimulants of protein kinase C (PKC), which is activated following hydrolysis of phosphatidylinositol-bis phosphate, stimulate secretion of β-endorphin from hypothalamus. We studied the separate effects of stimulants of PKC including phorbol myristate acetate (PMA), and oleolyl-acetylglycerol (OAG), a diacylglycerol analogue, on secretion of immunoreactive (IR-)β-endorphin (RIA) from dissociated fetal rat hypothalamic cell cultures. PMA (10^{-8}, 10^{-6} M) produced a dose-dependent stimulation of IR-β-endorphin release consisting of a peak IR-β-endorphin increment of approximately 50% over control secretion. OAG (10^{-8} M) also stimulated IR-β-endorphin secretion.

Conclusion: Hypothalamic β-endorphin secretion can be stimulated by activation of the phosphoinositol second messenger system, presumably at least via stimulation of PKC.

423.5

EFFECT OF SPECIFIC OPIATE RECEPTOR-SUBTYPE AGONISTS AND ANTAGONISTS ON THE RELEASE OF VASOPRESSIN AND OXYTOCIN. B.J.M. van de Heijning*, H. Rigter and Tj.B. van Wimersma Greidanus*. Rudolf Magnus Institute, Utrecht, The Netherlands.

Various endogenous opioid peptides affect the secretion of vasopressin (VP) or oxytocin (OT) from the neural lobe. As very specific (ant-)agonists to the opiate receptor subtypes μ, κ and δ are now available, the assessment of the subtype(s) involved in neurohypophyseal hormone release became possible. Male Wistar rats (200 g), 24 hr water-deprived, were injected sc and decapitated. Plasma levels of VP and OT were determined by RIA in trunk blood. No effect on either hormone was found 30 min after application of the δ-agonist DPDPE (dose 0.01-5 mg/kg). However, the μ-agonist DALDA (Schiller 1989, J.Med.Chem.32, 698) applied in the same dose range strongly inhibited the release of both VP and OT, an effect that was maximal 30-60 min after injection. The κ-agonist U-69,593 induced a similar effect: VP plasma levels even became undetectable. The time-response curve showed a maximal effect 30 min after application of the compound. Antagonism of endogenous opioids by the δ-antagonist naltrindole (dose 0.01-10 mg/kg) did not affect the release of either hormone, whereas both the κ-specific antagonist nor-binaltorphimine, and the relative μ-specific antagonist naloxone selectively enhanced OT plasma levels and not VP levels.

This indicates a selective and endogenous inhibition of the OT release mediated by κ-agonistic opioids (e.g. dynorphins) although μ-agonistic opioids might be involved as well. Furthermore, the κ- and μ-opioid receptors seem to play an important role in regulating the secretion of both VP and OT from the rat neural lobe.

423.6

EFFECTS OF ESTRADIOL ON MORPHINE-INDUCED CHANGES IN CYCLIC AMP ACCUMULATION IN NEUROBLASTOMA CELLS. A.Ratka[1], G.Hohchaus[2], J.W.Simpkins[1], Depts of Pharmacodynamics[1] and Pharmaceutics[2], University of Florida, Gainesville.

A phenotypically stable neuroblast subclone, SH-SY5Y, of the neuroblastoma cell line which express mu and delta opioid receptors was used. In this cell model the function of opioid receptor system was assessed by measuring cyclic AMP (cAMP) accumulation. Cells differentiated with retinoic acid (10uM) and treated with 1uM of prostaglandin E_1 (PGE_1) showed about 100 fold increase in cAMP level. When morphine (10uM) was given toghether with PGE_1, cAMP accumulation decreased significantly from 103.32±24.42 to 11.51±2.33 pmol/mg protein. In contrast to the acute effect of morphine 6 days of exposure to the opiate did not change the response of cells to the effect of PGE_1 on cAMP level. Further, chronic treatment with morphine prevented the inhibitory effect of acute morphine treatment on cAMP. Exposure of cells for 48 hrs to estradiol in doses of 5nM or 50nM did not change PGE_1-induced increase in cAMP accumulation. Moreover, in cells pretreated with either dose of estradiol, the suppressing effect of morphine was not changed. Treatment with 5nM of estradiol for 6 days potentiated cells response to PGE_1 from 103.07±15.82 to 178.24±16.53 pmolcAMP/mg protein. The inhibitory effect of morphine on cAMP was significantly less pronounced after 6 days than after 48 hrs exposure to estradiol (47.80±5.20 vs 25.76±3.21 pmol/mg protein of cAMP, respectively). We conclude that under in vitro conditions short-term treatment with estradiol has no effect, while chronic exposure to estradiol enhances the stimulatory effect of PGE_1 and reduces morphine-induced suppression of cAMP accumulation in cells which express mu and delta opioid receptors.

(Supported by AG 02021).

423.7

A NOVEL OPIOID CONTROL OF PROLACTIN SECRETION IN IMMATURE RATS S. Blackford and C. Kuhn. Dep't of Pharmacology, Duke University Medical Center, Durham, NC 27710.

The present study explores developmental changes in mu and kappa opiate receptor control of prolactin (PRL) secretion. The ontogeny of mu and kappa receptor function was determined by assessing the PRL response to the mu agonist sufentanil (SUF,.02 mg/kg, s.c.) and the kappa agonist U50488 (1 mg/kg, s.c.) in 5, 10, 15 and 20 day old rats. Both mu and kappa agonists stimulated PRL secretion at all ages. SUF action was selectively blocked by the mu antagonist beta-funaltrexamine and U50488 was selectively blocked by the kappa antagonist nor-binaltorphimine. Serotonin mediation of opiate-induced changes in PRL secretion was explored across ontogeny by testing cyproheptadine (CYPRO, 10 mg/kg) blockade of agonist responses in 5, 10, 20 and 60 day old rats. CYPRO attenuated the PRL response to the mu agonist SUF (.02 mg/kg, s.c.) in 20 and 60 day old rats, but not in the 5 or 10 day old pups. CYPRO did not block the kappa agonist U50488 (1 mg/kg s.c.) at any age. These results confirm the existence of a serotonin-mediated mu control of PRL secretion in older rats and a kappa control of PRL secretion that is independent of serotonin at all ages. Most interestingly, the results reveal a third control: an early developing serotonin-independent mu response. Supported by DA 02739.

423.8

Endogenous Opioid Regulation of Cell Proliferation in the Developing Rat Retina. T. Isayama, P.J. McLaughlin, and I.S. Zagon, Penn. State Univ. Coll. Med., Hershey, PA 17033.

The role of endogenous opioid systems in regulating cell proliferation in the developing retina was examined in 1-day-old rats. Met-enkephalin (MENK), naltrexone (NTX), or a mixture of MENK and naloxone (NALX) were administered to experimental animals; controls received sterile water. After three and one-half hours, animals were injected with 10 uCi/g of [^{3}H]thymidine and sacrificed one-half hour later. The labeling index of DNA-synthesizing cells within the germinative layer (GL) of the developing retina was determined from autoradiograms. A labeling index of 35.8% was obtained for control retinas. MENK-treated animals had a 10.6% decrease in the percentage of proliferating cells in comparison to controls, while an increase of 6.5% was seen in the NTX-treated retinas; MENK-NALX-treated retinas exhibited no change. Immunocytochemical preparations of the 1-day-old rat retina showed that MENK-immunoreactivity was abundant in the ganglion cell layer (GCL), and also present in the GL. These data suggest that opioids are present in the retina and act as natural inhibitory trophic factors to tonically govern cell replication in the developing eye.

Supported by NIH NS20500.

423.9

PRESENCE OF ZETA (ζ), A GROWTH-RELATED OPIOID RECEPTOR, IN RAT CEREBELLUM. I.S. Zagon, D. Gibo*, and P.J. McLaughlin. Penn. State Univ. College of Medicine, Hershey, PA 17033.

Interaction between endogenous opioids and opioid receptors have been shown to regulate cell proliferation in the developing rat brain. [Met^5]-enkephalin (ME) is a very potent endogenous peptide which serves as a trophic factor that inhibits cell replication. [^{3}H]-ME was used to probe the binding site of 6-day old rat cerebellum. Specific, high affinity, and saturable binding to [^{3}H]-ME was recorded in cerebellar homogenates; Kd=2.0±0.3 nM, Bmax=23.1±1.7 fmol/mg protein. Binding isotherms were linear with protein and dependent on time, pH, temperature, and a cocktail of protease inhibitors. Addition of Na+, Mg++, and guanyl nucleotides reduced binding; trypsin eliminated binding, suggesting a proteinaceous binding site. Competition studies using a wide variety of endogenous and exogenous opioids, including those selected for other opioid receptor subtypes, revealed that ME was the most selective ligand for the binding site. These results indicate the presence of a new opioid binding site, zeta (ζ), in the developing nervous system which is related to cell proliferation. Supported by NIH grant NS20500.

423.10

ENDOGENOUS OPIOID SYSTEMS ARE PRESENT AND REGULATE THE GROWTH OF BACTERIA. P.J. McLaughlin, W.J. Lutz*, and I.S. Zagon. Penn. State Univ. College of Medicine, Hershey, PA 17033.

Endogenous opioid systems are present in many different eukaryotes. Interaction of endogenous opioids and opioid receptors has been shown to tonically regulate growth of neural systems, with endogenous opioids acting as inhibitory trophic factors. Little information exists on opioids and the growth of prokaryotes. Using *Staphylococcus aureus* grown in liquid broth, effects of 10^{-6}M methionine enkephalin (ME), a potent opioid agonist, 10^{-6}M naltrexone (NTX), a potent opioid antagonist, or sterile water (CO), on growth of log phase cultures were determined. Turbidity readings (650_{OD}) and colony counts indicated that ME significantly depressed growth of *S. aureus*; naloxone blocked the inhibitory action of ME. NTX increased growth rates. Receptor binding assays demonstrated the presence of specific and saturable binding to [^{3}H]-ME; Kd=1.7 nM, Bmax=190 fmol/mg protein. Radioimmunoassays revealed ME in media after 24 hr in culture. Growth of bacterial strains such as *Pseudomonas aeruginosa* and *Serratio marcesans* also were regulated by endogenous opioid systems. These studies demonstrate the presence of endogenous opioids and opioid receptors in prokaryotic cells, and suggest that their interaction may regulate growth of bacteria. Supported by NIH NS20500 and NS20723, and the Pennsylvania Research Corp.

423.11

EFFECTS OF U- AND K-AGONISTS ON THE INTRACELLULAR FREE CALCIUM IN ISOLATED RAT MYOCYTES. X.D. Huang*, K. Tsou and T.M. Wong*. Department of Physiology, University of Hong Kong, Hong Kong.

U-(Wong et al. unpublished) and k-agonists (Wong and Lee, Neurosci Lett 77, 61-66, 1987) have been shown to induce arrhythmias in the isolated perfused rat heart. Since alterations in calcium fluxes are related to arrhythmogenesis, we studied the effects of these two agonists on intracellular free calcium (Ca_i) in isolated myocyte with a spectrophotometric method using fura-2 AM as the florescence indicator. Isolated myocytes were prepared from hearts of female Sprague Dawley rats of 100-150 g according to the method of Young and Scarpa (FEBS Lett, 223,53-58, 1987). U-agonists, D-ala^2, NMe^4, Gly-ol)-enkephalin (Peninsula lab.) and morphine and k-agonists, dynorphin$_{1-13}$ (Peninsula Lab.) and U50,488H (UpJohn Co.) increased Ca_i. The effect was blocked by their respective antagonists, naloxone (DuPont Pharmaceutical Co.) and MR 2266 (Boehringer Ingelheim), which did not produce any effect themselves. Chronic injection with morphine at increasing doses to rats according to the procedure of Wong and Lee (Neurosci Lett, 77,61-66, 1987) or addition of morphine (100 μM) to cultured isolated myocytes at 12 h before experiment also abolished the effect of morphine in elevating Ca_i. The results of the present study showed that μ- and k-agonists increase the Ca_i via opioid receptors on the membrane of myocytes. Such effect is probably responsible for their arrhythmogenic action in the isolated perfused rat heart. (Supported by a Croucher Foundation Grant to T.M.W.. MR 2266, naloxone and U50,488H were kindly supplied by Boehringer Ingelheim Co., DuPont Pharmceutical Co. and Upjohn Co., respectively).

423.12

EXPRESSION OF PROENKEPHALIN A PEPTIDES IN ADRENAL AND EXTRA-ADRENAL TISSUE OF FETAL AND ADULT RABBITS. J. Padbury, A. Martinez*, E. Burnell*, S. Thio*, D. Habib* and B. Chappell*. Harbor-UCLA Medical Center, Torrance, CA 90509.

The adrenal medulla contains large quantities of enkephalin (ENK) peptides. There is little developmental data on ENK peptides in adrenal or extra-adrenal tissue. We measured free MET-ENK, total ENK and CA in fetal adrenal (FAd) and fetal extra-adrenal tissue (FEAd) in rabbits (age 29 days, n=38) and adult adrenal (AAd, n=5). MET-ENK was measured by RIA, Total ENK after digestion with trypsin and carboxypeptidase B, and CA by radioenzymatic assay. ENK results are in ng/gland and CA in mcg/gland (Mean ± SEM).

	MET-ENK	Total ENK	MET/TOTAL	NE	EPI
(FAd)	2±.1	3±.3	.4±.03	.3±.04	.8±.07
(FEAd)	2±.3	3±.5	.5±.03	1±.01	.03±.0
(AAd)	22±.5	289±22	.07±.02	11±6	48±46

Conclusions:1) Fetal rabbit adrenal and extra-adrenal tissue have large amounts of proenkephalin derived peptides. 2) Post-translational processing (MET/TOTAL ratio) is significantly different in immature rabbits. Speculation: Extra-adrenal tissue is an important source of ENK in fetal and neonatal life.

423.13

EXPRESSION OF THE PREPROENKEPHALIN A GENE AND RELATED PEPTIDES IN HEARTS OF SPONTANEOUSLY HYPERTENSIVE RATS. M. Dumont, M. Ouellette*, L. Brakier-Gingras* and S. Lemaire. Department of Pharmacology, University of Ottawa, Ottawa, K1H 8M5, and *Département de Biochimie, Université de Montréal, Montréal, H3C 3J7.

The expression of the preproenkephalin A gene (Enk gene) was investigated in the heart of Wistar Kyoto (WKY) and spontaneously hypertensive (SHR) rats. The level of Met-Enk was measured by radioimmunoassay and the relative abundance of the Enk mRNA was determined by Northern blot analysis. WKY rats did not display any significant change in their cardiac Enk mRNA content with age but their cardiac Met-Enk levels (free and cryptic peptides) were significantly lower in 16 week old animals (from 1.5 to 0.9 pmol/g:free; from 5.2 to 3.7 pmol/g: cryptic). In SHR rats, the relative abundance of Enk mRNA in the heart increased with age to levels exceeding those found in WKY animals by factors of 2 and 4 fold at 8 and 16 week-old, respectively. The increase in 16 week-old SHR was localized in the ventricles. Cardiac free and cryptic Met-Enk in SHR rats did not increase with the age of the animal and were identical or lower (25% decrease in cryptic Met-Enk of 16 week-old SHR) than those found in WKY rats. The striking lack of parallelism between the ventricular levels of Enk mRNA and of free and cryptic Met-Enk suggests that the expression of the Enk gene into Enk-related peptides is suppressed at a translational level both in control and hypertensive animals. Supported by HSFO.

423.14

C-TERMINAL PROTEOLYSIS MODIFIES CARDIOREGULATION BY *B*-ENDORPHIN. M.D. Hirsch, A.E. Villavicencio*, J.E. McKenzie* and W.R. Millington. Physiol, USUHS, Bethesda, MD & Basic Life Scis., U Mo-KC, KC, MO.

C-terminal proteolysis profoundly alters activ-i-ty in the CNS analgetic system by converting the opioid receptor agonist *B*-endorphin(*BE*) 1-31 to the potent antagonist, *BE* 1-27. The present study examined effects of this processing in caudal medulla on central cardioregulation. The cisterna magna was exposed in rats prepared with adrenal vein and femoral artery catheters. Peptides (1.5 nmol) or CSF were infused intracisternally. At 30 min postinjection, baseline mean arterial pressure (MAP=94.7+-1.9mmHg) was reduced 24% by *BE* 1-31, and 32% by *BE* 1-27, but neither peptide altered heart rate (HR=366+-15beats/min). The peptides elicited differential compensatory increases in adrenal catecholamine release, with norepinephrine predominating for *BE* 1-31 (17-fold vs CSF), and with epinephrine for *BE* 1-27 (4-fold vs CSF). These findings underline the importance of regionally selective post-translational processing of *BE* 1-31 on functional specificity. Supporting Grants:USAMRDC 86PP6813 & NIDA DA04598

423.15

PRO-OPIOMELANOCORTIN (POMC) mRNA IN THE NUCLEUS TRACTUS SOLITARIUS AND OTHER EXTRAHYPOTHALMIC BRAIN REGIONS. D.M. Bronstein, M. K.-H. Schafer*, K. A. Trujillo, S. J. Watson and H. Akil, Mental Health Research Institute, University of Michigan, Ann Arbor, MI 48109.

While a variety of techniques have clearly established that the major group of POMC cell bodies in the CNS is situated in the arcuate nucleus of the hypothalamus, the presence of extrahypothalamic POMC cells in the CNS is less certain. A small diffuse cluster of POMC cell bodies has been localized in the nucleus tractus solitarius (NTS) area of the caudal medulla, using immunohistochemical methods, but POMC mRNA has never been detected in this region; conversely, while POMC mRNA-like signals have been found in a number of other extrahypothalamic brain sites (eg., amygdala, cerebral cortex), there is no anatomical evidence of POMC soma in these regions. In the present study, we used biochemical (Northern and RNase protection analyses) and anatomical (*in situ* hybridization) techniques to determine the distribution and regulation of POMC mRNA in extrahypothalamic brain regions. In addition to the arcuate nucleus, we detected POMC mRNA in the nucleus accumbens, caudate, and NTS by RNase protection assays using a riboprobe complementary to the 5' end of exon 3 of POMC mRNA. In contrast to previous reports that POMC mRNA in the amygdala or cortex is shorter than the arcuate message, we found no apparent difference in the size of the POMC message from different brain regions. Using *in situ* hybridization, POMC mRNA signal was detected in a small number of cells in the NTS following 1-2 months exposure. The number of POMC mRNA copies per cell appears to be lower in the NTS than in the arcuate nucleus. That we observed a relatively strong POMC mRNA-like signal in the caudate and nucleus accumbens with biochemical methods but no signal (to date) using *in situ* hybridization may indicate a diffuse distribution with low copy number/cell in these tissues. We are currently examining whether extrahypothalamic and arcuate POMC mRNA are identical species and whether they are regulated in similar ways. (Supported by NIDA DA02265 and NIMH MH422251 (S.J.W. & H.A.) and NIH training grants (D.M.B. & M.K.-H.S.)).

423.16

THE EFFECT OF CHRONIC MORPHINE ADMINISTRATION ON SPINAL ß-ENDORPHIN LEVELS. HB Gutstein,* DM Bronstein, and H Akil. Department of Anesthesiology and Mental Health Research Institute, University of Michigan, Ann Arbor, MI 48109

The effect of chronic opiate administration on endogenous opioid systems remains unclear. However, changes in opioids during the development of tolerance may play a role in some of the manifestations of dependence and withdrawal. We have been investigating the consequences of chronic exposure to morphine on the ß-endorphin (ßE) system in the CNS. ßE is a highly potent endogenous opioid which, when administered exogenously produces profound analgesia, tolerance, and dependence. Previous studies showed that chronic morphine administration had an inhibitory effect on rostral ßE systems (Bronstein, DM, Brain Res., 1990, in press). The present study was undertaken to see if spinal cord ßE was regulated similarly.

Sixty male Sprague-Dawley rats anesthetized with ether were implanted with a single morphine (75 mg) or placebo pellet on day 1, and 3 additional pellets of the same type on day 4. On day 7, rats were injected i.p. with either naloxone (2.5 mg/kg) or saline and sacrificed 1 hour later. ßE immunoreactivity (ßEIR) was determined for 3 different spinal cord regions by RIA.

Animals implanted with morphine showed significant increases in ßEIR in cervical and thoracic regions compared with controls; in the lumbosacral region this trend did not reach statistical significance. Acute naloxone injection had no significant effect on ßEIR. However, chronic naloxone in conjunction with chronic morphine has not yet been tested. Analysis of peptide forms is currently underway.

Our results showing an increase in spinal ßEIR with chronic morphine administration appear to differ from observations in more rostral CNS systems. Spinal ßE is mainly derived from the nucleus tractus solitarius, while brain ßE mainly arises form the arcuate nucleus. This suggests that these two discrete endorphinergic systems may be regulated differently. These findings may have important implications for understanding the intrinsic mechanisms resulting in narcotic tolerance and dependence.

423.17

PERIPHERAL EFFECTS OF VARIOUS OPIOID AGONISTS ON NEUROGENIC EXTRAVASATION IN SKIN OF THE RAT HIND PAW. A. Barber*, R. Gottschlich* and A.F. Haase. Pharma Research, E. Merck, Frankfurter Strasse 250, D-6100 Darmstadt, FRG.

Several reports have indicated that peripheral opiate receptors may mediate some of the analgesic actions of opiates. To address this question, we examined plasma extravasation in rat hind paw skin as a model of neurogenic inflammation. Cut saphenous nerves were stimulated (5 mA, 1 msec, 2 Hz, 10 min) antidromically in anaesthetised, Evans Blue-treated rats. Extravasation was determined by measuring the level of dye in the skin after extraction. Drugs were administered i.v. Morphine and the κ agonist (-)U50488H produced a dose-dependent inhibition (ID_{50} 2.4 and 0.68 mg/kg, respectively) of plasma extravasation. The action of 5 mg/kg morphine and (-)U50488H was completely reversed by 1 mg/kg naloxone, which is indicative of an opiate-mediated effect. (+)U50488H was ineffective at 1 mg/kg, and produced only 35 % inhibition at 5 mg/kg, thus demonstrating the stereoselectivity of this effect. 5 mg/kg of the δ/μ agonist DADLE reduced extravasation by 64 %, whereas the δ agonist DPDPE and the σ ligand (+)SKF 10047 were inactive at this dose. The results presented are therefore consistent with the view that μ and κ, but not selective δ or σ agonists, can inhibit neurogenic inflammation in the skin. Whether this effect is mediated by peripheral opiate receptors on nerve endings of primary afferent fibres, or an indirect systemic action of opiates remains to be determined.

OPIOIDS: ANATOMY AND PHYSIOLOGY II

424.1

EXTRA- AND INTRACELLULAR ELECTROPHYSIOLOGICAL PROPERTIES OF OPIOID SENSITIVE NEURONS IN THE BED NUCLEUS OF THE STRIA TERMINALIS. **M. Dalsass and A. Siegel, Neurology, V.A. Medical Center, E. Orange, N.J. 07019, and Dept. of Neurosciences, N.J. Medical School, Newark, N.J. 07103.**

The bed nucleus of the stria terminalis (BNST) plays a critical role in the modulation of aggressive behavior. This modulatory function is also directly influenced by opioid peptides administered to the BNST. Our aim was to examine how BNST neurons respond to opioid peptides and their antagonists in order to understand, at the cellular level, the mechanisms of action of these substances.

Rats were anesthetized with chloral hydrate (400 mg/kg) and recordings were obtained from BNST neurons using either single or multibarrel pipettes. Responses to D-Ala^2-Met^5-enkephalinamide (DAME) [10mM] and naloxone hydrochloride (50 mM) were examined. Extracellular responses of BNST neurons following microiontophoretic application of DAME showed predominantly excitatory effects, with increasing (>50%) firing rates above baseline levels. These responses were blocked by naloxone. More rarely, inhibitory responses could be elicited by DAME and these showed variable responsiveness to naloxone. Our initial findings utilizing intracellular recordings suggest a complex interaction of opioid peptide and antagonist on both the resting membrane potential and the duration of the spike discharge. [Supported by NIH grant NS 07941-20 and the UMDNJ Foundation].

424.2

DOPAMINE OVERFLOW IN N. ACCUMBENS FOLLOWING OPIOIDS AND ELECTRICAL BRAIN STIMULATION AS ASSESSED BY MICRODIALYSIS IN FREELY MOVING RATS. Margaret E. Hamilton and Agu Pert. BPB/NIMH, Bethesda, MD. 20892.

Previous investigation has demonstrated a failure of morphine to increase extracellular levels of striatal dopamine (DA) in conscious rats. In contrast, other research has indicated that the activity of VTA - n. accumbens DA neurons and concomitant DA release may be increased by opioid agonists. Typically, increased DA neurotransmission in the n. accumbens is associated with enhanced locomotor activity. Acute systemic administration of morphine to naive rats produced only moderate, biphasic increases in DA overflow in the medial n. accumbens. Initially, the sedative effect of morphine prevailed. As this effect subsided, however, a slight increase in DA was observed, accompanied by renewed behavioral activity as measured by photocell counts. Pretreatment of rats with the DA uptake inhibitor, nomifensine, attenuated both the sedative phase and the second mild DA increase; however the increases in DA by morphine were proportionally similar to those in rats that did not receive nomifensine. Microinjection into the VTA of the met-enkephalin analogue, DALA, also elicited behavioral activation and moderate DA overflow in the n. accumbens. Similarly, electrical stimulation of the VTA with intermittent trains of unipolar pulses (150 μsec width, 20 Hz at 250 μA) increased both measures in a manner similar to the effects of opioids. It appears, therefore, that merely a relatively modest elevation of DA in the n. accumbens may be required to enhance behavioral activation. Alternatively, non-DA systems presumably originating in the VTA and perhaps involving endogenous opioids may contribute significantly to behavioral arousal.

424.3

ELECTROPHYSIOLOGICAL EFFECTS OF OPIOIDS ON NEURONS IN THE VENTRAL TEGMENTAL AREA. S.W. Johnson and R.A. North. Vollum Inst., Oregon Health Sci. Univ., Portland, OR 97201.

Dopamine (DA) and opioid peptides were superfused onto ventral tegmental area (VTA) neurons in slices cut horizontally from rat brain. Intracellular recordings were made from 53 cells: 74% were hyperpolarized by DA (1-100 μM) and 19% were hyperpolarized by methionine-enkephalin (ME, 0.1-30 μM). None were hyperpolarized by both DA and ME. Effects of ME were mimicked by DAMGO (0.3-3 μM), but not by DPDPE (3 μM) or U50488H (3 μM), suggesting activation of mu receptors. Electrophysiological properties of DA- and ME-responsive cells resembled primary and secondary neurons, respectively, described in the substantia nigra compacta (J. Neurosci. 9:1233-1241, 1989). Electrical stimulation of the slice rostral to the VTA evoked synaptic potentials (SP's) that were partly blocked by APV (30 μM) and CNQX (10 μM), and partly blocked by picrotoxin (100 μM). ME reduced both the glutamate and GABA components of the SP by 30-40%, but only at high concentrations (30 μM). Spontaneous SP's, evoked by raising K^+ to 6.5 or 8.5 mM, were depolarizing with KCl-filled electrodes, hyperpolarizing with acetate-filled electrodes, and blocked by bicuculline (30 μM). ME (0.3-3 μM) reduced the frequency of SP's by more than 50% in 2 of 3 neurons. We conclude that opioids act at mu receptors to hyperpolarize GABA interneurons thus reducing inhibitory input on DA-responsive neurons in the VTA.

424.4

SYSTEMIC METHIONINE ENKEPHALIN (ME) INCREASES THE EXTRACELLULAR NOREPINEPHRINE CONCENTRATION IN THE ROSTRAL VENTROLATERAL MEDULLA (RVLM) OF THE RAT. P.A. MASON[1], J.A. STRICKLAND[2], S. LEE*[2] and H.M. RHEE[2]. Depts. of Anatomy, Cell Biology and Neuroscience[1] and Pharmacology[2], Oral Roberts University, Tulsa, OK 74171.

Our objective was to determine if the CNS was involved in the hypotensive effect of systemic ME. We used microdialysis to determine the effects of systemic ME on the extracellular concentration of monoamines in the RVLM, a central site of cardiovascular regulation. Rats were anesthetized and the femoral artery and vein of each rat were cannulated. A dialysis probe was implanted in the RVLM and perfused for 2 hr to obtain baseline data. The rat then received a bolus injection of saline (300 μl IV) followed 40 min later by a bolus injection of ME (100 μg/kg/300 μl IV). At the end of each experiment, glutamate (0.16 mg/10 μl/5 min) was perfused through the probe to verify indirectly the probe's placement in the RVLM. ME decreased BP without a reflex-mediated increase in heart rate. HPLC with EC detection showed that ME significantly increased the NE concentration but did not change the concentrations of DOPAC, HVA and 5-HIAA. Saline had no effect. Glutamate increased the MAP from 87 to 103 mm Hg. These results are consistent with the concept that systemic ME decreases BP via altering the NE concentration in the RVLM.

424.5

OPIOID AFFERENTS TO THE LOCUS COERULEUS FROM THE ROSTRAL MEDULLA AS DETECTED BY RETROGRADE TRANSPORT COMBINED WITH IMMUNOHISTOCHEMISTRY. G. Drolet, H. Akaoka, E.J. Van Bockstaele, G. Aston-Jones and M.T. Shipley[1], Div. Behav. Neurobiol., Dept. Mental Health Sci., Hahnemann U., Philadelphia, PA 19102 and [1] Dept. Anat. Cell Biol., U. Cincinnati Coll. Medicine, OH 45267.

The nucleus locus coeruleus (LC) is densely innervated by fibers immunoreactive for opioid peptides. However, the origin(s) of these inputs has not been determined. In the present study, we combined retrograde transport and immunohistochemical techniques to identify the source of enkephalin (Enk) inputs to the LC. The retrograde tracer, cholera toxin B coupled to colloidal gold particles (CTB-gold), was injected into the electrophysiologically identified LC nucleus. After survival for 5-7 days, the rats received colchicine (100 μg, icv) and were perfused 24 hours later. Results for retrograde labeling with injections restricted to LC confirmed previous reports that major afferents to LC derive from the nucleus paragigantocellularis (PGi) in the rostral ventrolateral medulla, and the mediodorsal prepositus hypoglossi (PrH) in the dorsomedial medulla. Interestingly, the distribution of Enk cells in the PrH was confined to the mediodorsal portion which contains LC afferents. In the ventrolateral medulla the Enk cells were aggregated into groups, including one in the retrofacial portion of PGi which contains LC projecting neurons. Examination of Enk and gold labelling in the same tissue sections revealed numerous doubly-labeled cells in both PGi and PrH. These enkephalinergic pathways from the rostral medulla could represent an anatomical substrate underlying opioid effects on LC neurons during stress, opiate abuse and tolerance.
Support: PHS grants NS 24698 and DA 06214 and Fonds de la Recherche en Santé du Québec.

424.6

LOCAL, NALOXONE-PRECIPITATED WITHDRAWAL IN THE VENTROLATERAL MEDULLA ACTIVATES LOCUS COERULEUS NEURONS VIA AN EXCITATORY AMINO ACID PATHWAY. H. Akaoka, G. Drolet, C. Chiang and G. Aston-Jones. Div. Behav. Neurobiol., Dept. Mental Health Sci., Hahnemann University, Philadelphia, PA 19102

Noradrenergic neurons of the nucleus locus coeruleus (LC) in morphine-dependent rats are strongly activated by opiate-withdrawal (OW). We examined possible mechanisms for such LC activation using extracellular recording of LC unit activity in halothane anesthetized rats.

There was no evidence that hyperexcitability of LC neurons caused their activation during OW, as chronic morphine (i) did not affect the activation of LC cells by iontophoretic glutamate (11/13), and (ii) reduced the excitation of LC cells by sciatic nerve stimulation (by 27%, $p<0.05$, n=31).

Possible involvement of the ventrolateral medulla (VLM; containing the nucleus paragigantocellularis, the major afferent to LC) in OW-activation of LC was examined with local injections of the opiate antagonist naloxone (NLX), to induce anatomically-restricted OW. NLX microinjected into VLM (10^{-2} M, 500 nl) significantly activated LC neurons (2.3 fold, 24/35) in dependent rats. This effect is specific (i) to dependent rats (3/27 in naive rats), (ii) to opiate receptors as the inactive enantiomer, +NLX, failed to mimic the effect of racemic NLX (n=5), and (iii) to VLM as NLX injections rostral to active site in the same animal (n=5) or in LC (n=3) were ineffective. Smaller doses of NLX injected into VLM produced only weak excitations of LC (10^{-6} and 10^{-4} M, n=14). The activation of LC cells by local or systemic NLX was blocked by kynurenate (an antagonist of excitatory amino acids, EAAs; 10^{-2} M) directly infused into LC (n=3). These results indicate that VLM may be the primary site whereby OW activates LC neurons and that this activation is produced by an EAA input to LC. Supported by PHS grants NS 24698 and DA 06214 and FRSQ.

424.7

TRANSIENT DESENSITIZATION OF μ-OPIOID RECEPTORS IN RAT LOCUS COERULEUS. G.C. Harris and J.T. Williams. Vollum Inst., Oregon Hlth. Sci. Univ., Portland, OR 97201.

The noradrenergic nucleus, the locus coeruleus (LC), contains a high density of opiate receptors, and has been proposed to play a role in mediating opiate withdrawal responses. Acutely, both μ-opioid agonists and α_2-adrenergic agonists decrease the spontaneous activity and hyperpolarize LC neurons by increasing a potassium conductance. Experiments were conducted using intracellular recording in rat brain slices. The hyperpolarization induced by [Met^5]enkephalin (ME) (300 nM) was tested before and after a 5 min application of ME (30 μM). The hyperpolarization induced by the low concentration of ME was decreased by 84% immediately after washout of the higher concentration. The amplitude of the initial hyperpolarization completely recovered after approximately 20 mins. This desensitization appeared to be primarily homologous for the μ-opioid receptor because the actions of α_2-agonists were only marginally depressed. Pretreating cells with forskolin, isobutyl methylxanthene, or 8-bromo-cAMP had no effect on the acute response to opioids or on the acute desensitization response. Pretreatment with the kinase inhibitor, staurosporin, significantly reduced the receptor desensitization but did not completely block it. This acute response could represent the initial stages in the development of opiate tolerance. Supported by USDHHS DA 04523 and DA 05387.

424.8

REGULATION OF OPIOID PEPTIDE AND RECEPTOR SYSTEMS FOLLOWING EXOGENOUS OPIOID ADMINISTRATION IN NEONATAL RAT BRAIN. A. Tempel. Dept. of Psychiatry, Laboratory of Neuropharmacology, Hillside Hospital/LIJMC, P.O. Box 38, Glen Oaks, New York 11004

Opioid analgesics are well known to produce tolerance and dependence in vivo and desensitization in vitro, although the mechanisms for these phenomenon are not clear. We have found that chronic pre- and postnatal morphine treatment of rat pups produces a significant decrease in brain μ opioid receptor density with no change in receptor affinity. This downregulation is accompanied by tolerance to the actions of morphine. In order to determine changes in specific brain regions, autoradiography was performed on brain sections from control and chronic morphine treated pups. Four days of postnatal morphine treatment induced a 30% loss of μ opiate receptors in the patches of the striatum with no significant loss in the matrix area. This loss of μ receptors was no longer observed with prolonged morphine treatment. These data provide evidence for a unique plasticity of the immature opiate receptor system. In order to determine if chronic morphine treatment causes alterations in opioid peptide synthesis, preproenkephalin (PPE) mRNA levels were examined in brains of control and chronic morphine treated animals. Chronic morphine treatment produced a significant decrease in PPE mRNA levels relative to saline treated animals in various brain regions. Data from these studies provide insight into interactions between opioid gene expression and brain function in opiate tolerance and dependence in the developing central nervous system.
(Supported by NIDA grant DA-05440).

424.9

MU TOLERANCE IN ONTOGENY: ANTINOCICEPTIVE STUDIES. R. Windh and C. Kuhn. Dep't of Pharmacology, Duke University Medical Center, Durham, NC 27710.

The opiate receptor's capacity to adapt is one important determinant of the physiologic outcome following developmental opiate exposure. To examine this question, the present study evaluated tolerance at mu opiate receptors in developing rats using an antinociceptive model. Animals were treated for 5 days with the agonist morphine (5mg/kg-25mg/kg s.c., twice daily) on days 4-8 or 22-26 and challenged 48 hours later with sufentanil (1.5-8ug/kg in 10 day olds; 2-14ug/kg in 28 day olds). Tolerance was determined by measuring the shift in the sufentanil dose-response curves in chronic morphine and saline treated rats. Sufentanil administration to opiate-naive 10 and 28 day old rats caused a dose-dependent and naloxone reversible increase in latency to paw removal from a hot plate. Chronic treatment had markedly different effects at the two ages studied: while the 28 day olds showed no antinociceptive response to any dose of sufentanil, the dose-response curve in 10 day olds was unchanged. These results show that mu receptors involved in antinociception show a relative inability to adapt to chronic perturbation early in development. This resistence to tolerance contrasts with our previous demonstration of tolerance in corticosterone secretion, suggesting there may be a spectrum of adaptability at various sites. However, both these findings are consistent with a literature indicating less robust tolerance in developing animals, and they suggest that opiates might exert more profound effects on developing animals than on adults after chronic administration. Supported by DA 02739.

424.10

PARTICIPATION OF COERULOSPINAL NORADRENERGIC PATHWAY IN FENTANYL-INDUCED MUSCLE RIGIDITY IN THE RAT. P.W. Lui, T.Y. Lee* and S.H.H. Chan. Department of Anesthesiology, Veterans General Hospital-Taipei and Institute of Pharmacology, National Yang-Ming Medical College, Taipei, Taiwan, Republic of China.

This study further examined our previously identified role of coerulospinal noradrenergic pathway in fentanyl-induced muscle rigidity, based on a combined histochemical, immunocytochemical and pharmacologic evaluation. Unilateral, site-specific microinjection of fentanyl (2.5 µg/50 nl) into the locus coeruleus (LC) of Sprague-Dawley rats anesthetized with ketamine evoked a significant increase in the electromyographic activity recorded from the caudal lateral extensor (CLE) muscle. This correlate of opiate-induced muscle rigidity was antagonized by a pretreatment with the specific α_1-adrenoceptor blocker, prazosin (250 µg/kg, i.v.). The above results were significantly eliminated in animals pretreated with the selective noradrenergic neurotoxin , DSP4 (50 mg/kg, i.p.). There was also an appreciable reduction in the number of nerve terminals that stained positively with antiserum against dopamine-β-hydroxylase, and were impinged on CLE motoneurons identified by horseradish peroxidase labelling. These data further confirmed that the coerulospinal noradrenergic pathway may be directly involved in the elicitation of muscle rigidity by fentanyl, possibly via α_1-adrenoceptors in the spinal cord.

424.11

INTRARENAL EFFECTS OF μ-OPIOID AGONIST DERMORPHIN IN RATS. D.R. Kapusta*, S.Y. Jones* and G.F. DiBona. Dept. of Int. Med., Univ. Ia. Col. Med. & VAMC, Iowa City, IA 52242

Changes in renal function were examined during left renal artery (lra) infusion of the selective μ-opioid agonist, dermorphin, in anesthetized Sprague-Dawley rats with intact (n=6) and bilaterally denervated kidneys (DNX; n=7). Urine was collected from left and right (control) kidneys via ureteral cannulae during 20 min. lra infusion periods of isotonic saline control, dermorphin (0.5 nmol/min/gKW), and isotonic saline recovery for measurements of urine flow rate (V) and urinary sodium excretion ($U_{Na}V$). Results: Left renal artery dermorphin did not alter mean arterial pressure, heart rate, right kidney V or $U_{Na}V$. Values are left kidney means ± S.E. per gKW; * P < 0.05 vs control.

	Intact		DNX	
	V	$U_{Na}V$	V	$U_{Na}V$
	µl/min	µeq/min	µl/min	µeq/min
Control	11.1±1.8	2.14±0.51	8.3±1.4	1.80±0.63
Dermorphin	7.3±1.4*	1.46±0.49*	12.1±2.5	2.06±0.71
Recovery	8.2±1.3	1.65±0.44	10.8±2.1	2.28±0.54

Left kidney glomerular filtration rate and renal plasma flow were similar before and during lra dermorphin (0.9±0.2 vs 0.8±0.1 ml/min/gKW and 3.0±0.4 vs 2.6±0.3 ml/min/gKW), respectively. These results suggest that μ-opioid agonists participate in the renal tubular sodium and water reabsorption via an intrarenal action to facilitate the nerve terminal release of norepinephrine.

424.12

EFFECTS OF OPIOID AGONISTS AND ANTAGONISTS ON GABA-INDUCED CURRENTS IN ISOLATED GOLDFISH RETINAL BIPOLAR CELLS. H.-J. Du* and A. Kaneko. National Institute for Physiological Sciences, Okazaki, 444 Japan.

Various kinds of neuropeptides have been found in amacrine cells of the vertebrate retina, but their physiological roles are unknown. In the present study, effects of different opioid peptides on isolated goldfish retinal bipolar cells were investigated by using the whole-cell voltage clamp recording technique. The opioids tested include Met- & Leu-enkephalins, selective (μ-, δ- & κ) or non-selective opioid receptor agonists and antagonists, in isolation or in combination. All drugs (0.01-50 μM) were delivered through a rapid superfusion system. The amplitude of the GABA-induced Cl^--currents (GABA response) was reduced to 21.4 % - 88.8 % of the control by any one of opioid peptides (at 50 μM). The depressive effect developed gradually, reached the maximum in 3-5 min, and persisted for >5 min. μ-, δ- & κ-agonists depressed the GABA response in the same cell. Opioid antagonists were unable to block or reverse these effects. In contrast, they did cause similar, even stronger, depression in a dose-dependent manner (ED_{50} around 1×10^{-7} - 10^{-6}M). These effects were reversible. In some cases, a synergic action was seen between the agonist and antagonist. The present data indicate a multiple opioid receptor-mediated modulation of signal transmission at the bipolar cells and complications of the action of opioid agonist and antagonist on a single isolated cell. Supported by the Fellowship from the Japanese Ministry of Education, Science and Culture to H.-J. Du. (Present address of Du: Shanghai Brain Research Institute, Shanghai, 200031 China.)

424.13

SIGMA AGENTS DO NOT MODULATE SUCROSE REINFORCEMENT. R.B. Murphy, L.H. Schneider, J. Davis, J. Gibbs, and G.P. Smith, Bourne Lab, NY Hospital-Cornell Med Ctr, White Plains, NY 10605, Dept Chemistry, N.Y.U., NY, NY 10003, and Dept. Psychology, Univ. Illinois-Chicago, Chicago IL 60680

Occupancy of the sigma receptor/binding site has been suggested to modulate the ascending dopamine (DA) systems on the basis of the localization of sigma receptors adjacent to nigrostriatal DA cell bodies (Graybiel et al., 1989) and the behavioral actions of selective sigma agents as putative neuroleptics (Taylor et al., 1988). We utilized microstructural analysis of lick patterns to examine the actions of the selective sigma agents [(+)-SKF 10,047, 5 mg/kg; BMY 14802, 4 mg/kg], and compared them with haloperidol (0.1 mg/kg), which has high affinity for both DA and sigma receptors. Six male S-D rats with chronic gastric fistulas were adapted to 30-min tests sham feeding a 20% sucrose solution after an 18h food deprivation and injection i.p. at -15 min. We observed that there was no effect of either selective sigma drug on volume sham fed. In contrast, haloperidol produced a highly significant reduction in sham intake (33.7 ± 2.7 ml to 15.0 ± 2.7 ml; $p<.001$). However , BMY 14802, but not SKF 10,047 or haloperidol, produced a significant decrease in interlick intervals from 0-250 msec ($p<.005$) as well as in the number of licks per mL consumed ($p<.05$). No other microstructural changes were produced by the selective sigma agents; the effects of haloperidol on other microstructural measures confirmed our previous studies (Schneider et al., 1989). These results suggest that sigma receptors do not modulate dopaminergic systems which are critical to the reinforcing potency of sucrose. Supported by NIMH RSA MH00149 (GPS), NIH R29 NS24781 (LHS), and NIDA RO1 DA 05728 (RBM).

424.14

SIGMA LIGAND PENTAZOCINE (PENT) INCREASES EXCITABILITY IN HIPPOCAMPAL NEURONS. A.E. Cole, J.J. Aryanpur, C.U. Eccles and R.S. Fisher. Dept. of Neurol., Johns Hopkins Hospital, Baltimore, MD 21205 and Dept. of Pharm. and Toxicol., Univ. of Maryland School of Pharmacy, Baltimore, MD 21201.

The sigma receptor is a novel site implicated in psychosis and epilepsy. The sigma receptor ligand PENT has been shown to increase field potential amplitude at low concentrations (1-10 uM) in the in vitro hippocampal slice (Eccles et al., Neurosci. Abstrs., 1989). The present study examined the effect of PENT on intracellularly recorded synaptic responses in hippocampal pyramidal neurons of region CA1. In the presence of 10 uM PENT the amplitude of the EPSP evoked by orthodromic stimulation of the Schaffer collateral pathway increased to 150% of control values. No significant effects were observed on the resting membrane potential or input resistance. At the same time, the IPSP was preserved in all cells examined. In additional neurons where KCl-filled electrodes were used, PENT appeared to increase both the frequency and amplitude of spontaneous IPSP's. In all experiments PENT was applied by bath perfusion; APV and naloxone were added to block potential interactions with NMDA and opiate receptors, respectively. These results demonstrate that PENT increases synaptic excitability of hippocampal neurons. The data suggest a physiological action of sigma receptors on synaptic function in rat hippocampus.

425.1

THYROTROPIN-RELEASING HORMONE (TRH) mRNA IS INCREASED IN SPECIFIC LIMBIC SUBREGIONS FOLLOWING ELECTROCONVULSIVE SEIZURES (ECS) AS DETERMINED BY IN SITU HYBRIDIZATION HISTOCHEMISTRY (ISHH). K.S. Fuson, M.R. Aydelotte,* A. Sattin and M.J. Kubek. Depts. of Anatomy, Psychiatry and Program in Medical Neurobiology, Indiana University & VA Medical Centers, Indianapolis, IN 46202.

ECS produces marked and sustained (days) elevations of TRH in specific CNS regions (Ann. N.Y. Acad. Sci. 553:286,1989). The possibility that ECS might induce TRH mRNA in a similar fashion was examined using ISHH. Male S-D rats were given ECS or sham ECS every other day for a total of 3 treatments, then decapitated at 3, 6, 12, & 24 hrs following the last seizure. Frozen sections (20 µm) were hybridized with a 35S-CTP labeled 548b TRH riboprobe at 60C for 20hrs. Film (48 hr, -70C) and emulsion (14d, 4C) were used for evaluation via computer image analysis. Increases in TRH mRNA were seen in hippocampal dentate gyrus at 3, 6 and 12 hrs, with a maximum occuring at 6 & 12 hrs, whereas less hybridization was seen in CA regions. Significant increases were found in piriform ctx at 6 & 12 hrs. No hybridization was seen in similar regions of the sham groups. These ECS studies are the first to show: 1) Induction of TRH mRNA in specific regions previously shown to respond with high levels of TRH; 2) TRH mRNA is not normally expressed in these same regions; 3) TRH mRNA is expressed in a unique temporal manner in given areas; and 4) Elevations of TRH in limbic foci are the result of de novo synthesis. Supported by NS 25661 and VA.

425.2

MODULATION OF LIMBIC SEIZURE INDUCED ELEVATIONS IN TRH CONTENT BY MK-801. M.S. Kreider and A. Winokur. Dept. of Psychiatry, Univ. of PA, Phila., PA 19104.

Previous studies in our laboratory have shown that induction of limbic seizures by kainic acid (KA) produced large increases in the concentration of TRH in limbic regions of the rat CNS (Kreider et al, Regul. Pep. 28:83-93, 1990). These increases are differentially attenuated by diazepam pretreatment (Wolfinger et al, Neurosci. Abs. 14:399, 1988). We investigated whether the NMDA antagonist MK-801 would prevent KA induced elevations in TRH content.

Male Sprague-Dawley rats (180-200g) received i.p. injections of MK-801 (5 mg/kg) or saline. Thirty minutes later, rats received KA (12 mg/kg) s.c. or saline. All rats were sacrificed 3 days later and regional TRH content determined by radioimmunoassay.

MK-801 prevented the KA induced increase in TRH in the corpus striatum and posterior cortex. KA-induced elevations in dorsal hippocampus were attenuated by MK-801, while elevations in ventral hippocampus were not affected. In the amygdala/piriform cortex, KA induced elevations in TRH were enhanced by MK-801. These results indicate that there is a differential effect of MK-801 on the responsiveness of TRH systems to limbic seizure activity.

425.3

VASOACTIVE INTESTINAL PEPTIDE (VIP) IN THE NORMAL AND DECENTRALIZED SUPERIOR CERVICAL GANGLION (SCG). H.Hyatt-Sachs, M.C. Beinfeld+, C. Baldwin and R.E. Zigmond, Department of Neurosciences, Case Western Reserve University, Cleveland, OH 44106 and +Department of Pharmacology, St. Louis University, St. Louis, MO 63104

The rat SCG has been shown by immunohistochemical techniques to contain VIP-like immunoreactive (VIP-IR) neural processes and a small number of immunoreactive cells (J. Comp. Neurol. 280:522, 1989). Double-labeling studies have demonstrated that VIP-IR is also present in cell bodies of preganglionic sympathetic neurons that project to the SCG (Soc. Neurosci. Abst.14:355, 1988). Finally, ligation of the preganglionic cervical sympathetic trunk led to the accumulation of VIP-IR both on the spinal cord side and on the ganglion side of the ligature. These data indicate that some, but not all, of the VIP-IR in the SCG is present in preganglionic nerve fibers. In the present study, VIP-IR was determined by RIA of acetic acid extracts of SCG. The lyophilized extract was assayed for VIP-IR using a VIP antiserum from Peninsula Laboratories and yielded a dilution curve that was parallel with authentic VIP. The content of VIP-IR in SCG from normal rats was 11.6 pg/SCG (112.5 pg per mg extracted protein; N=26 ganglia). When the SCG extract was run on HPLC, VIP-IR was detected only in those fractions corresponding to the retention time of authentic VIP. VIP-IR was also measured in the SCG 48h after cutting the cervical sympathetic trunk bilaterally. The content of VIP-IR was increased by 2-fold in decentralized ganglia compared to ganglia from both unoperated and sham-operated animals. Examination of sections of the SCG six weeks after decentralization revealed an increase in the number of immunostained neuronal processes and no apparent change in the number of immunostained neuronal cell bodies. These data suggest that there is an increase in VIP in other afferent inputs to the SCG or, possibly, in postganglionic neuronal processes following section of the preganglionic nerve trunk (NS12651, NS18667 and MH00162).

425.4

THE PROBABLE IDENTITY OF A NEUROKININ METABOLIZING ENZYME WITH THE LYSOSOMAL "PROTECTIVE PROTEIN". H.L. Jackman, F. Tan,* H. Tamei,* C. Beurling-Harbury,* X.-Y. Li,* R.A. Skidgel,* E.G. Erdös* Lab. of Peptide Research, Dept. of Pharmacology, Univ. of Ill., Coll. of Med., Chicago, IL 60612

Most of the actions of neurokinins depend on an intact C-terminal amide (Met-NH_2). We extracted an enzyme from human platelet granules that deamidates substance P and other neurokinins. The enzyme, released from human platelets by thrombin, was purified to homogeneity. The purified deamidase has a molecular weight of 52kDa and consists of 2 chains, a 33 kDa and a 21 kDa chain. The enzyme is a serine protease; [^{3}H]-DFP labels the active site serine on the heavy chain. The purified enzyme has esterase, peptidase and deamidase activities. With biologically active peptides, the enzyme acts both as a deamidase (substance P, neurokinin A and eledoisin) and as a carboxypeptidase (bradykinin, angiotensin I) at neutrality, although the carboxypeptidase action is faster at pH 5.5. After sequencing the first twenty-five amino acids of both chains, an identical sequence was found in the corresponding two chains of lysosomal "protective protein." A defect in this protein is the cause of galactosialidosis, a severe genetic disorder with progressive neurologic deterioration and mental retardation. (These studies were supported by NIH: HL36082, HL36473, HL36081, DK41431.)

425.5

CALCIUM-BINDING PROTEINS IN APLYSIA NEURONS. A. Hermann (1), T.L. Pauls*(2) and C.W. Heizmann*(3). (1) Univ. of Salzburg, Dept. of Zoophysiology, A-5020 Salzburg, Austria; (2) Univ. of Zürich-Irchel, Inst. of Pharm. and Biochem., CH-8057 Zürich; (3) Univ. of Zürich, Dept. of Pediatrics, CH-8032 Zürich, Switzerland.

Protein pattern of single, identified neurons (bursting, beating and silent) within the nervous system of the marine mollusk, Aplysia californ. were studied by 2D-Page. Major differencies between neurons were found in the range of low molecular weight and low isoelectric point, where Ca-binding proteins are generally located. Comigration experiments revealed a prominent protein group for bursting R-15 cells which migrated at an identical position to carp II parvalbumin (PV), whereas a protein spot characteristic for silent R-2 cells migrated at a position close to crayfish sarcoplasmatic Ca-binding protein (SCP). Western blot analysis did not show immunocrossreactivity in the molecular range where PV-like proteins are found. Immunocrossreactivity was observed, however, with a different protein at Mr 40 000, pI 4.8. The same protein also crossreacted with an antiserum directed against rat calbindin D-28K and in addition bound high amounts of ^{45}Ca ions as revealed by transblot/Ca-overlay technique. The results suggest that this protein is a novel Ca-binding protein sharing common antigenic determinantes for both, PV and calbindin D-28K.

In heat treated extracts of ganglia we further identified a group of proteins with Mr 13000-20000, pI 4.6, immunocrossreactive to antibodies against Amphioxus SCP II that also bound high amounts of ^{45}Ca ions. These proteins which share a high degree of similarities to SCPs therefore appear to represent isomeric forms of these proteins in neurons. Supported by EMBO-fellowship ASTF 5696 and the Swiss National Science foundation (31-9409.88).

425.6

DETECTION OF THE C-TERMINAL PENTAPEPTIDE OF THE NEUROTENSIN PRECURSOR IN RAT. K. Muraki, S.P. Mitra, R.E. Carraway and S.E. Leeman. Physiology Dept., Univ. of Mass. Medical Center , Worcester, MA 01655.

A radioimmunoassay was developed towards the C-terminal pentapeptide (rCTP, sequence ASYYY) of the rat neurotensin (NT)-precursor using ^{125}I-labeled rCTP and rabbit antisera towards rCTP conjugated to succinylated thyroglobulin. The assay was sensitive to 4 fmol/tube and crossreacted <0.01% with NT, neuromedin N, xenopsin and GSYYY. Measured in acid/acetone extracts of adult SD rats, the level of rCTP (pmol/g, mean ±SD) was 5-10% that of NT in brain (rCTP, 0.5±0.1; NT, 6.8±1.4) and ileum (rCTP, 5.0±1.4; NT, 94±15), whereas it was 30-50% in colon (rCTP, 2.4±0.5; NT, 4.3±0.3) and pituitary (rCTP, 3; NT, 10). During reverse-phase HPLC 3-4 major forms of brain and intestinal rCTP activity were observed, 15-40% of which behaved like synthetic rCTP. Sucrose gradient centrifugation of isotonic homogenates of rat intestinal mucosa showed that rCTP activity co-banded with NT in the region containing secretory vesicles. These findings indicate that a substance chromatographically and immunochemically similar to rCTP exists in rat CNS & intestine along with other related peptides not yet identified. Supported by Fogarty 5PW04213 and NIH DK28565.

425.7

EFFECT OF FLUNARIZINE ON THE METHAMPHETAMINE- AND 3,4-METHYLENEDIOXYMETHAMPHETAMINE-INDUCED CHANGES IN EXTRAPYRAMIDAL NEUROTENSIN. J.W. Gibb, G.R. Hanson, L.G. Bush*, K Mitros* and M. Johnson. Dept. Pharmacol. and Toxicol., University of Utah, Salt Lake City, UT 84112.

We recently observed that flunarizine (FLU) protects serotonergic systems from the neurotoxic action of 3,4-methylenedioxymethamphetamine (MDMA). The purpose of this study was to determine if administration of FLU also influenced methamphetamine (METH)- or MDMA-induced changes in extrapyramidal neurotensin systems. Male Sprague-Dawley rats (180-250 g) were injected with 4 doses of METH (15 mg/kg, s.c.), MDMA (10 mg/kg, s.c.) or saline at 6-h intervals with or without prior FLU (30 mg/kg, i.p.) administration. The animals were killed 18 h after the last drug administration. FLU administration alone resulted in a 36% increase in striatal neurotensin-like immunoreactivity (NTLI) while having no effect on the nigral levels. METH and MDMA increased striatal NTLI to 187% and 184% of control, respectively; nigral concentrations were increased to 579% and 441%, respectively. FLU treatment potentiated the METH and MDMA responses in the striatum while failing to alter the nigral changes. The responses are similar to the effects of a dopamine D2 receptor antagonist on the METH-induced changes, thus suggesting that FLU affects D2 receptor transmission. This conclusion is supported by the observation that FLU increases dopamine metabolites 3 h after a single administration. These results demonstrate that serotonergic and peptidergic changes resulting from MDMA tretament are differentially mediated. (Supported by USPHS grants DA 00869 and DA 04222)

425.8

ROLE OF DOPAMINE AND GLUTAMATERGIC SYSTEMS IN THE 3,4-METHYLENEDIOXYMETHAMPHETAMINE-INDUCED CHANGES IN BRAIN NEUROTENSIN AND DYNORPHIN A. M. Johnson, L.G. Bush*, J.W. Gibb and G.R. Hanson. Dept. Pharmacol. and Toxicol., University of Utah, Salt Lake City, UT 84112.

The role of the dopaminergic and glutamatergic systems in the 3,4-methylenedioxymethamphetamine (MDMA)- induced increase in rat brain neurotensin-like immunoreactivity (NTLI) and dynorphin-like immunoreactivity (DLI) levels was examined. Peptide content was measured 18 h after a single dose (10 mg/kg, s.c.) of the drug. MDMA increased neostriatal concentrations of NTLI and DLI to 274% and 366% of control, respectively. Nigral concentrations of NTLI and DLI were increased to 269% and 308%, respectively, while the levels in the nucleus accumbens were increased to 145% and 181% of control, respectively. Coadministration of the dopamine D1 receptor antagonist, SCH 23390 (0.5 mg/kg, i.p.), prevented the MDMA-induced increase of NTLI and DLI in all three brain structures while the administration of the dopamine D2 receptor antagonist, sulpiride (80 mg/kg, i.p.), failed to alter the MDMA effects except for a slight attenuation in the increase of neostriatal DLI. Blockade of the N-methyl-D-aspartate (NMDA) receptor complex using the noncompetitive inhibitor, MK-801 (1 mg/kg, i.p.), prevented the MDMA effects on both peptides in the neostriatum, nucleus accumbens and substantia nigra. These findings suggest that MDMA-induced changes in the neurotensin and dynorphin systems involve both the dopaminergic D1 and the glutamatergic NMDA receptors.(Supported by USPHS grants DA 00869 and DA 04222)

425.9

SECRETION OF CARBOXYPEPTIDASE H FROM AtT-20 PITUITARY TUMOR CELLS. D.Parkinson. Dept. Cell Biology and Physiology, Washington University School of Medicine, St. Louis, MO 63110.

The biosynthesis of peptide transmitters requires the proteolytic processing of precursor by several enzymes. In order for these enzymes to function, they must be packaged along with the precursor into the secretory vesicles where the appropriate milieu is maintained. Carboxypeptidase H (CPH) removes C-terminal basic amino acids from partially-processed precursor. To gain insights into the mechanism of sorting of proteins into the secretory vesicle, the secretion of CPH has been investigated in AtT-20 mouse pituitary tumor cells.

AtT-20/D-16v cells were grown to near-confluence in DME supplemented with 10% Nu-Serum. Media was collected and analyzed for CPH content by enzyme assay or western blotting. AtT-20 cells secrete enzymically-active CPH constitutively. The accumulation of CPH activity was linear over a 10 hour period. This activity was stimulated 8-10 fold by cobalt ions and potently inhibited by CPH-selective inhibitors. CPH secretion was stimulated 3-5 fold by secretagogues. There was a direct correlation between CPH activity and the staining of a 54kD band on western-blotting with an antibody directed to the C-terminal sequence. These results show that CPH is not processed at its C-terminus. To test the importance of acidic compartments in the secretion of CPH, cells were treated with ammonium chloride. This treatment produced small increases in secreted CPH activity. This result suggests that constitutively-secreted CPH is active, regardless of whether it has passed through any acidic compartments.

425.10

METABOLISM OF EXTRACELLULAR N-ACETYLASPARTYL-^{3}H-GLUTAMATE AND ITS PRODUCT ^{3}H-GLUTAMATE IN INTACT CELLS OF MURINE WHOLE BRAIN PRIMARY CULTURE. **M. Cassidy and J. H. Neale Dept. of Biology, Georgetown University, Washington DC 20057**

N-Acetylaspartylglutamate (NAAG) may function in synaptic communication in the mammalian CNS. It is present in synaptic vesicles and released on stimulation in several systems, yet the extracellular fate and effect of this peptide remain unclear. The goal of this study is to define the extracellular metabolism of NAAG and the fate of both NAAG and glutamate in intact cells. Primary cultures of murine brain were incubated with N-acetylaspartyl-^{3}H-glutamate in the presence and absence of inhibitors of NAAG peptidase activity. Data suggest that NAAG is rapidly degraded by a cell-surface enzyme to N-acetylaspartate and ^{3}H-glutamate. Initial results indicate that in a whole cell system, ^{3}H-glutamate, and to a much lesser extent, intact ^{3}H-NAAG, are taken up by cells. This suggests removal of NAAG from the medium by both degradation and limited direct uptake. The role of NAAG in communication may be two-fold: as a rapidly acting neurotransmitter or a chronic source of glutamate in the synaptic cleft, serving to modulate neuronal excitability.

425.11

BIOTINYLATED EGG-LAYING HORMONE: BIOLOGICALLY ACTIVE AND INACTIVE FORMS. S.L. Knock, B.T. Miller, A. Kurosky*, G.T. Nagle, J.E. Blankenship. Marine Biomed. Inst., Dept. Anat. & Neurosci., and Dept. Human Biol. Chem. & Genetics, Univ. Tex. Med. Br., Galveston, TX 77550.

Aplysia egg-laying hormone (ELH) was chemically modified using an N-hydroxysuccinimide (NHS)-Long Chain (LC)-Biotin reagent. Sites of modification were analyzed by FAB-MS, HPLC, amino acid compositional analysis and protein sequence analysis. Several species of biotinylated ELH were produced, owing to the variable reactivity of the N-terminal α-amino group and the two ε-amino groups of Lys^8 and Lys^{36}. The ELH-Lys^{36}-Biotin species was found to be biologically active and equipotent to native ELH in an egg-laying bioassay and in an in vitro neuroassay using cell R15 and identified left lower quadrant neurons in the abdominal ganglion. Analysis of the reactivity of the primary amines indicated that Lys^{36} was significantly more reactive than Lys^8 or the N-terminus. These results are significant for two reasons. First, this is the first report of a chemically labeled, biologically active ELH molecule. With the development of biotinylated-ELH, it should be possible to pursue studies in receptor binding and target tissue localization. Second, lower reactivity of the N-terminal amino group suggests that it may not be as accessible as the ε-amino group of Lys^{36}. This reduced access could be associated with a mechanism that renders the peptide less susceptible to aminopeptidases and, therefore, could contribute to an increased lifetime for ELH in the animal.

425.12

INDUCTION OF NEUROPEPTIDE Y SYNTHESIS IN RAT EPILEPSY MODELS. M.Ortler[1]*, G.Sperk*, J.Marksteiner*, R.Bellmann*, and R.Widmann[2]* (SPON.: Europ. Neurosci. Ass.). Depts. of [1]Histology and Pharmacology, Univ. Innsbruck, Austria and [2]Max-Planck-Inst. Neuronal Sci., Köln, FRG.

Recently we observed marked increases in brain levels of neuropeptide Y (NPY) and NPY-mRNA after kainic acid (KA) induced seizures in the rat. By measuring incorporation of locally injected ^{3}H-tyrosine, now we found a markedly enhanced (by about 350%) biosynthesis rate of NPY in the cortex 2 to 30 days after KA. We furthermore investigated changes of NPY immunoreactivity and gene expression after KA and after pentylenetetrazol kindling. In both animal models pronounced increases of NPY immunoreactivity were observed in the terminal field of mossy fibers. In KA treated rats NPY immunoreactivity extended to the molecular layer of the dentate gyrus after 30 to 60 days, suggesting neuronal sprouting. Unilateral injection of colchicine into the hilus abolished NPY staining in the the mossy fibers. Using "in situ" hybridization, in both animal models markedly enhanced expression of prepro-NPY-mRNA was observed in the granular layer. It is suggested that sustained expression of the neuromodulatory peptide NPY, in addition to the observed plastic changes, may contribute to altered excitability of hippocampal mossy fibers in epilepsy. Somatostatin immunoreactivity and gene expression were not changed in mossy fibers or granule cells, respectively.

425.13

INDUCTION OF NEUROKININ B BUT NOT OF NEUROKININ A BIOSYNTHESIS IN THE HIPPOCAMPUS IN EPILEPSY OF THE RAT. G.Sperk*, J.Marksteiner*, M.Ortler[1]*, O.Hornykiewicz[2] and R.Wahler*. Depts. of Pharmacology and of [1]Histology, Univ. Innsbruck, 6020-Innsbruck and Institute of [2]Biochem. Pharmacology, Univ. Vienna, 1090-Vienna, Austria.

Recently we observed marked increases in brain levels of neurokinin B in the frontal cortex and hippocampus after kainic acid induced seizures and after pentylene=tetrazol kindling in the rat. Using immunohistology and "in situ" hybridization we now investigated changes in neurokinin B immunoreactivity and in mRNA encoding for neurokinin A and B, respectively. In both animal models we observed pronounced increases of neurokinin B immunoreactivity in granule cells, in the terminal field of mossy fibers and in pyramidal cells of the CA1 sector. According to this also markedly enhanced expression of prepro-neurokinin B mRNA was observed in the granular layer as well as in pyramidal cells of the CA1 sector. Enhanced neurokinin B immunoreactivity and prepro-neurokinin B mRNA were also found in the frontal and pyriform cortices (mainly in layer 2). Prepro-neurokinin A mRNA was present in high concentrations in neurons of the habenula, but only in minute amounts in the hippocampus. It was unchanged in epileptic rats. Our experiments suggest marked induction of neurokinin B but not of neurokinin A gene expression in both animal models of temporal lobe epilepsy.

425.14

PRODUCTION AND DIFFERENTIAL ENDOCRINE REGULATION OF ATRIAL NATRIURETIC PEPTIDE (ANP) IN NEURON-ENRICHED PRIMARY CULTURES. C.F.Deschepper, K.P.T.Nguyen*, M.C.LaPointe*, K.R.Zahs and D.G. Gardner*. Depts of Physiology and Metabolic Research Unit*, Univ. of California, San Francisco CA 94143.

Neuron-enriched primary cultures were obtained from the brains of fetal rats. Ten days after plating, the cultures were incubated in serum-free medium and the amounts of ANP secreted into the medium was assessed by RIA. More ANP was secreted by neuronal cells derived from fetal day 17 rats vs. day 16, by cells from diencephalon vs. cortex and by cells at 39°C vs. 37°C. ANP mRNA transcripts were identified in cytoplasmic extracts from neuronal cultures by S_1 nuclease protection and shown to have a similar transcription start site as that employed by rat atrium and fetal hypothalamus in vivo. Pulse-label analysis of newly synthetized ANP in neuronal cultures revealed that, unlike neonatal cardiocyte cultures, the majority of the secreted immunoreactive material migrated with processed forms of ANP (approx. 3kD) rather than the prohormone (approx. 14kD). In contrast to earlier findings with rat atrial cardiocytes, dexamethasone and triiodothyronine decreased ANP secretion and ANP mRNA concentration in cultured neurons. This suggests the possibility of tissue-specific differences in the regulation of ANP gene expression by exogenous hormones.

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425.15

ISOLATION AND CHARACTERIZATION OF *DROSOPHILA* PEPTIDES CONTAINING AN -ArgPheNH$_2$ C-TERMINUS. R. Nichols and M. Conkright*. Departments of Biological Chemistry and Biological Sciences, University of Michigan, Ann Arbor, MI 48109.

Naturally-occurring *Drosophila* peptides containing -ArgPheNH$_2$ C-terminal sequences have been isolated and characterized. Purification of peptides from crude homogenates were monitored utilizing a radioimmunossay specific for -ArgPheNH$_2$. Various chromatographic methods based on size and charge were used to purify the peptides. Drosulfakinin-I (DSK-I) and three peptides from pro-FMRF have been isolated. A novel peptide was also identified. The structures of the purified peptides were determined by automated Edman degradation and fast-atom bombardment mass spectrometry (FABMS).

A number of other immunoreactive peaks have been identified and their structures are being characterized. It is anticipated that many of the peptides will correspond to previously unreported forms of DSK, FMRFamide or related peptides.

SEROTONIN III

426.1

MDMA-TREATED MONKEYS CONTINUE TO SHOW BRAIN SEROTONERGIC DEFICITS EIGHTEEN MONTHS AFTER DRUG TREATMENT. M. B. Martello, A. L. Martello, J. L. Katz and G. A. Ricaurte. Dept. of Neurology, Johns Hopkins Univ. School of Medicine and NIDA Addiction Research Center, Baltimore, MD 21224.

Monkeys administered (±)3,4-methylenedioxymethamphetamine (MDMA) show large (75-86%) depletions of regional brain serotonin (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) two weeks later (JAMA: 260: 51-55, 1988). By ten weeks, partial recovery takes place such that 5-HT and 5-HIAA are depleted by only 30-47% (Neurosci. Abs. 14: 558, 1988). The purpose of the present study was to determine if serotonergic recovery in the MDMA-treated primate continues over time and, if it does, to ascertain whether it is complete by 18 months.

Six squirrel monkeys (Saimiri sciureus) were administered MDMA hydrochloride s.c. at a dose of 5 mg/kg twice daily (0800 and 1700 hrs.) for 4 days. Three of these animals were killed 7 months later; the remaining 3 were sacrificed 18 months later. Regional brain 5-HT and 5-HIAA determinations were then performed using HPLC-EC methods, with the two treatment groups being assayed in parallel. Relative to untreated controls (n=3), MDMA-treated monkeys at both survival periods (7 and 18 months) showed substantial reductions in both 5-HT and 5-HIAA (32-70% depletion, depending on marker and brain region). Notably, there was no evidence of recovery between 7 and 18 months.

These results suggest that 5-HT neural damage induced by MDMA in the primate is long-lasting, possibly permanent. Further, they suggest that the partial recovery of 5-HT which takes place in the monkey occurs largely within the first few months after MDMA exposure. Given that the regenerative capacity of central 5-HT neurons in the primate appears to be limited, these findings could have important public health implications since human use of MDMA continues. [Supported by DA05707 and in part by MAPS]

426.2

EVALUATION OF THE NEUROTOXIC POTENTIAL OF 2-HYDROXY-4,5-METHYLENEDIOXYMETHAMPHETAMINE (2-HOMDMA), A REPORTED METABOLITE OF MDMA. Z. Zhao,[1] G. Ricaurte[2] and N. Castagnoli, Jr.[1] Dept. of Chemistry, Virginia Tech, Blacksburg, VA 24061 and [2]Dept. of Neurology, Johns Hopkins University School of Medicine, Baltimore, MD 21224.

The serotonergic neurotoxin MDMA [2-methylamino-1-(3,4-methylenedioxyphenyl)propane] is reported (Lim and Foltz, Soc. Tox. Abs. #1333, Feb. 1990) to be metabolized to 2-HOMDMA, a possible precursor to the corresponding "6-hydroxydopamine analog", a potentially neurotoxic agent. The synthesis and full characterization of this MDMA metabolite as a racemate have been accomplished via regiospecific Vilsmeier formylation of 3,4-methylenedioxyphenol followed by conversion of the resulting 2-hydroxy-4,5-methylenedioxybenzaldehyde as the protected benzyl ether to the corresponding nitrostyrene derivative which in turn was reduced to the primary amine with $LiALH_4$. Reaction of this amine with ethyl formate followed by $LiALH_4$ reduction of the resulting formamide and hydrogenolysis to remove the benzyl protecting group led to the desired product which was obtained as a stable, crystalline hydrochloride salt. Systemic administration of 2-HOMDMA to male Sprague-Dawley rats at a dose 4 times greater than that of MDMA required to cause a 60-70% depletion of regional brain serotonergic markers one week later was without effect. Similar results were observed following intraventricular administration of 2-HOMDMA, using 5,7-dihydroxytryptamine as a positive control. These results argue that the neurotoxicity of MDMA may not be linked to its conversion to a 6-hydroxydopamine type metabolite.

426.3

EFFECTS OF MDMA ON HIPPOCAMPAL AND BRAIN STEM MAO ACTIVITY

E.T. Kokotos and E.C. Azmitia. Dept. of Biology, New York Univ., New York, N.Y. 10003

Monoamine oxidases are mitochondrial-bound enzymes which break down monoamines such as serotonin (5-HT) in the nerve cell. Pargyline, clorgyline and yohimbine are drugs which inhibit MAO and have been used as mood-elevating drugs in the treatment of depression. 3,4-methylenedioxymethamphetamine (MDMA) is a designer drug which produces euphoria, and has been shown to affect 5-HT both by potentiating release and blocking uptake. The effects of MDMA on MAO activity in 5-HT terminals were studied to determine whether MDMA could also potentiate the above-mentioned effects by acting as an MAO inhibitor.

The hippocampus and brain stem from adult rat and young rats were removed and placed in 10x v/w 0.01M PB. Following homogenization and centrifugation, the remaining tissue was resuspended in 0.01M PB and incubated with ^{3}H-5-HT_0 and MDMA in a range of concentrations (10^{-4} - 10^{-6}M) at 37°C. MAO activity was measured by the scintillation counting of ^{3}H-5-HIAA, a degradation product of serotonin. An IC_{50} of $6x10^{-5}$M was found on MAO activity in both the hippocampus and brain stem of these animals. There were no differences in sensitivity to the drugs between sexes or between young rats and adult rats. The MAO inhibitory effects of this drug may account for the potentiation of its mood-elevating properties.

426.4

5-HT_2 ANTAGONISTS BLOCK THE NEUROTOXICITY OF METHYLENEDIOXYMETHAMPHETAMINE (MDMA) BY INTERFERING WITH STIMULATED DOPAMINE SYNTHESIS. C.J. Schmidt, V.L. Taylor* and G.M. Abbate. Merrell Dow Research Institute, Cincinnati, OH 45215.

5-HT_2 receptor antagonists have been shown to prevent the long-term deficits in 5-HT concentrations resulting from the administration of a single dose of MDMA to rats. In acute studies, both ritanserin and MDL 11,939 (α-phenyl-1-(2-phenylethyl)-4-piperidine methanol) attenuated the increase in striatal dopamine (DA) 3 h after MDMA. This increase in DA concentrations is due to a stimulation of synthesis following carrier-mediated efflux of DA as shown by its sensitivity to nomifensine but not haloperidol. To determine if the protective effect of the 5-HT_2 antagonists was due to this interference with stimulated DA synthesis, we administered the DA precursor L-DOPA (and carbidopa) with MDMA plus the antagonists. L-DOPA alone significantly potentiated the decrease in 5-HT measured 1 week after the administration of MDMA. More importantly, L-DOPA reversed the protective effect of both antagonists. The data are consistent with the hypothesis that 5-HT_2 antagonists block MDMA-induced neurotoxicity by interfering with MDMA-stimulated DA synthesis and that sustained DA release itself is required for MDMA-induced neurotoxicity.

426.5

REGENERATION IN THE CNS FOLLOWING MDA-INDUCED NEUROTOXICITY: PHARMACOLOGIC EVIDENCE FOR SELECTIVE REINNERVATION BY *FINE* SEROTONERGIC AXONS M.A. Wilson and M.E. Molliver Dept. of Neuroscience, Johns Hopkins Univ. School of Medicine, Baltimore, MD 21205.

The psychotropic amphetamine derivative 3,4-methylenedioxyamphetamine (MDA) causes degeneration of one class of serotonergic (5-HT) axon terminals, *fine axons*, but spares another class of 5-HT axon terminals, *beaded axons*. The denervation induced by MDA is followed by progressive reinnervation. The newly formed 5-HT axons exhibit morphologic features of fine axons; in order to assess their pharmacologic properties, we evaluated their vulnerability to a second course of MDA treatment. Rats received MDA, 20 mg/kg s.c., twice daily for 4 days; eight weeks later, half of the treated rats were given MDA again, according to the same protocol; all rats were sacrificed two weeks later. There is a substantial recovery of 5-HT-immunoreactive axon density in the forebrain two months after a single course of MDA. Two weeks after a second treatment with MDA, many of the newly formed 5-HT axons are lost; however, the loss is less severe than that observed two weeks after a single course of MDA. Thus, while most of the regenerated 5-HT axons are vulnerable to the effects of MDA, some appear to be resistant. Alternatively, accelerated regeneration may have occurred after the second MDA treatment, due to the "priming" effects of the first lesion. These results provide further evidence that after MDA-induced degeneration there is *regenerative sprouting* of fine 5-HT axons, rather than collateral sprouting of beaded axons. (Support: NIH DA04431, NS15199)

426.6

DIRECT MORPHOLOGIC EVIDENCE FOR DEGENERATION OF SEROTONERGIC AXONS FOLLOWING ADMINISTRATION OF AMPHETAMINE DERIVATIVES: dl-p-CHLOROAMPHETAMINE, d-METHAMPHETAMINE, AND d- and l-FENFLURAMINE.

Karen J. Axt, Thea M. Teune*, and Mark E. Molliver. The Johns Hopkins University School of Medicine, Baltimore, MD 21205.

The loss of markers for 5-HT innervation of forebrain caused by psychotropic amphetamine derivatives has suggested that these compounds are neurotoxic. To obtain direct evidence for axon degeneration, we examined the morphologic effects of these drugs at short survival times. Rats were injected with the following drugs: pCA 10 mg/kg x 2 days; d-MA 15 mg/kg x 5 or 100 mg/kg x 1; d- or l-FEN 5 or 25 mg/kg b.i.d. x 4 days. 2-3 days after the last dose, immunocytochemically-stained sections revealed numerous 5-HT axons with cytopathologic features that were not seen in control brains. Dilated 5-HT axon *terminals* with markedly swollen varicosities were observed within most terminal fields of rat forebrain, e.g., cerebral cortex. Structurally abnormal *preterminal* 5-HT axons exhibited increased caliber, multiple irregular pleomorphic dilatations, and engorged stump-like endings. Damaged preterminal axons were present in several fiber bundles including MFB, fornix, cingulum, striae terminalis & medullaris, and in layers I & VI of neocortex. All of the drugs tested induced similar pathologic changes which are indicative of axonal degeneration. The same pathology has been reported following exposure to methylenedioxyamphetamine (O'Hearn et al., 1988). Structural evidence for degeneration is observed only transiently: damaged 5-HT axons are most abundant 2-3 days after drug administration; by 1-2 weeks their incidence is greatly reduced. Based on this and previous data, the morphologic changes produced by neurotoxic amphetamines can be divided into 4 temporal phases; I: *Depletion* (hours), II: *Degeneration* (days), III: *Denervation* (weeks), and IV: *Reinnervation* (months). [Support: DA 04431 & PMAF]

426.7

SPECIFIC REGIONAL PATTERNS OF SEROTONERGIC INNERVATION IN VENTRAL AREAS OF FOREBRAIN IN THE RAT; SELECTIVE DENERVATION AND REINNERVATION AFTER ADMINISTRATION OF P-CHLOROAMPHETAMINE (PCA). Mark E. Molliver and Karen J. Axt. Dept. of Neuroscience, The Johns Hopkins University School of Medicine, Baltimore, MD 21205.

The 5-HT innervation of neocortex exhibits regional specificity in terms of density and laminar distribution of axons. Two 5-HT axon types, *fine & beaded*, have dissimilar distributions and differential vulnerability to psychotropic amphetamines. This study examines the 5-HT innervation, PCA-induced denervation, and reinnervation in selected allocortical areas and in the amygdaloid nuclei of the rat. In the olfactory tubercle, fine & beaded 5-HT axons form an exceptionally rich 5-HT innervation. Piriform cortex [PIR] has a dense plexus of fine 5-HT axons in layer I & III, but few in layer II. All areas of insular cortex exhibit many fine axons in layer I along with a zone containing both axon types in layers II-III. All layers of entorhinal cortex are richly innervated, but a prominent patch of beaded axons is limited to layers II-III of the lateral portion [LEA]. Nuclei in the amygdala can be delineated by distinctive patterns of 5-HT innervation. Following administration of PCA, which selectively ablates fine 5-HT axons, there is marked denervation with a pattern of axonal sparing unique to each zone. While PIR is markedly denervated, the patch of beaded axons remains in layers II-III of LEA. Selective axon loss in the amygdala leaves a dense 5-HT innervation in the basolateral nucleus [BL]. Nine months after PCA, much reinnervation of these areas by fine 5-HT axons has occurred, in advance of that in parieto-occipital areas of neocortex. The early reinnervation of these ventral telencephalic areas is ascribed to their proximity to preterminal axons in the MFB. The selectivity of sprouting appears to restore the original 5-HT innervation pattern. The specific patterns of innervation in these forebrain areas supports differential functional effects of the raphe nuclei in particular regions. [Support: DA04431 & PMA]

426.8

P-CHLOROAMPHETAMINE-INDUCED NEUROTOXICITY IN RAT BRAIN DEPENDS ON THE PRESENCE OF ENDOGENOUS SEROTONIN.

U.V. Berger, R. Grzanna and M.E. Molliver. Department of Neuroscience, Johns Hopkins University School of Medicine, Baltimore, MD 21205.

Systemic administration of p-chloroamphetamine (PCA) causes acute release of serotonin (5-HT) and produces long-lasting degeneration of 5-HT axons in rat brain. However, direct intracerebral injection of PCA does not produce toxic effects on 5-HT axons, suggesting that a neurotoxic compound is generated in the periphery following systemic administration. This study was designed to test the possibility that endogenous serotonin (5-HT) plays a role in mediating the neurotoxic effects of PCA in the brain. PCA was systemically administered to rats that had been previously depleted of 5-HT by different regimens of p-chlorophenylalanine and/or reserpine. Two weeks later, the serotonergic innervation of the forebrain was evaluated using immunocytochemical and biochemical methods. The results show that acute depletion of 5-HT storage pools provides substantial protection against the PCA-induced degeneration of 5-HT axon terminals. While drug interactions may possibly have a protective effect, it is more likely that the neurotoxicity of PCA is dependent on the release of endogenous 5-HT. The results show further that the protective effect is most profound following treatment regimens that are sufficient to deplete peripheral as well as central stores of 5-HT. We interpret this finding as evidence that peripheral stores of 5-HT, i.e., in platelets and enterochromaffin-cells, are necessary for the expression of PCA-induced toxicity. Based on these results, we propose that neurotoxicity is not produced directly by PCA itself but may be caused by a toxic metabolite of 5-HT. This mechanism may also account for the neurotoxicity seen with other psychotropic amphetamines such as 3,4-methylenedioxymethamphetamine. [Support: USPHS Grant DA-04431]

426.9

ACUTE AND CHRONIC EFFECTS OF THE α-ETHYL HOMOLOGUE OF *p*-CHLOROAMPHETAMINE (PCA). X. Huang*, M. P. Johnson, R. Oberlender, J. F. Nash and D. E. Nichols. Depts. of Pharmacology and Toxicology, and Medicinal Chemistry and Pharmacognosy, School of Pharmacy and Pharmacal Sciences, Purdue Univ., W. Lafayette, IN 47907. Dept. of Psychiatry, Case Western Reserve Univ., Cleveland, OH 44106.

Previous work from our laboratory has indicated that extension of the α-side chain in several substituted amphetamines attenuates the DA but not the 5-HT actions of the compounds. This report describes the effects of α-side chain extension in the PCA analog 1-(4-chlorophenyl)-2-aminobutane (CAB). The effects of CAB and PCA on the uptake inhibition of ^{3}H-5-HT and ^{3}H-DA, the extracellular concentrations of DA and DOPAC using *in vivo* microdialysis, behavioral activity as determined utilizing a two-lever drug discrimination paradigm with rats trained to discriminate 1.75 mg/kg *S*-MBDB, and the neurotoxicity of CAB as measured by HPLC-EC and ^{3}H-paroxetine binding parameters one week after a single dose were examined.

CAB was found to inhibit the uptake of both ^{3}H-5-HT and ^{3}H-DA with IC_{50} values of 331 ± 18 and 2343 ± 12 nM, respectively. This is 2.5-fold less potent than PCA (132 ± 7 nM) for ^{3}H-5-HT and 5-fold less potent than PCA (466 ± 15 nM) for ^{3}H-DA uptake inhibition. Similarly, CAB was found to be behaviorally less potent than PCA. The ED_{50} values (95 % confidence limits) were 0.17 (0.10 - 0.30) and 0.62 (0.41 - 0.93) mg/kg for PCA and CAB, respectively. CAB at 22 mg/kg, but not 11 mg/kg, significantly increased extracellular DA and decreased DOPAC in the striatum 3 hrs following administration. PCA (10 mg/kg) markedly increased extracellular DA and decreased DOPAC during the first 3 hrs after administrtion. Similarly, 5-HT, 5-HIAA and ^{3}H-paroxetine binding were significantly decreased one week following PCA or 22 mg/kg of CAB. Therefore, it appears that extension of the α-side chain of PCA attenuates but does not abolish the dopaminergic effects. In addition, the relative neurotoxicity and DA effects of PCA and CAB support the importance of DA in the serotonin neurotoxicity of PCA-like compounds.

426.10

COMPARISON OF THE EFFECTS OF REPEATED ORAL VERSUS SUBCUTANEOUS d,l-FENFLURAMINE ADMINISTRATION ON BRAIN SEROTONIN NEURONS IN THE RAT. S. Culp*, R. Zaczek, N.M. Appel, J.F. Contrera and E.B. De Souza, NIDA/ARC, Baltimore, MD 21224; FDA, Rockville, MD 20857

Repeated systemic administration of high doses of fenfluramine, a prescribed anorectic agent, produces dose-dependent decreases in brain serotonin (5-HT) markers characteristic of toxic actions of the drug. In view of the differences in the prescribed dose, route of administration, kinetics and metabolism of fenfluramine in humans versus rats, the relevance of the high-dose toxic effects of systemically administered fenfluramine in rats to the therapeutic use of the drug is unclear. The importance of the route of drug administration (oral vs s.c.) on the neurochemical effects and pharmacokinetics of repeated d,l-fenfluramine administration in rats (1-24 mg/kg b.i.d. 4 days) was examined. Overall, comparable, dose-dependent alterations in brain 5-HT markers were observed following oral and s.c. administration of fenfluramine. Doses of 1 and 2 mg/kg fenfluramine were without significant effects on 5-HT uptake sites. Higher doses of fenfluramine (4-24 mg/kg) produced dose-dependent decreases in 5-HT, 5-hydroxyindoleacetic acid and 5-HT uptake sites with maximal decreases (80-90%) occurring at 12 mg/kg. In brain, significantly higher levels of fenfluramine were observed following s.c. than following oral administration of the drug. In contrast, comparable levels of its active metabolite norfenfluramine were present in brain following the two treatments. These data suggest that norfenfluramine levels in brain may be more important in determining the high-dose neurotoxic effects of fenfluramine in rats. Furthermore, the data underscore the importance of species differences in metabolic profiles and pharmacokinetics of the drug and its metabolite in determining the relevance to humans of the high-dose toxic effects of fenfluramine in rats.

426.11

DL-FENFLURAMINE AND HIPPOCAMPAL SEROTONIN RELEASE IN THE AWAKE RAT. K.E. Sabol, J.B. Richards, and L.S. Seiden. Dept. Pharmacol. Physiol. Sci., Univ. Chicago, Chicago, IL 60637

Acute injections of D-fenfluramine cause increases in extracellular serotonin (5HT) in cortex, hypothalamus and n.accumbens in anesthetized rats (LaFerrere and Wurtman, 1989). DL fenfluramine increases cortical 5HT in freely moving rats 24 hours after dialysis probe implantation (Carboni and Di Chiara, 1989). In the present experiment, the effect of DL-fenfluramine on extracellular serotonin in the hippocampus in freely moving rats was studied. Rats were permanently implanted with BAS 4 mm dialysis probes (N=5). Fifteen hours later (Day 1) the rats were perfused with Ringers solution (0.20 ul/min) and basal 5HT levels were determined with HPLC-EC. 4.0 mg/kg DL-fenfluramine was then injected IP and perfusate was collected for an additional 3 hours. This procedure was repeated on Days 5 and 10 after probe implantation. The probes were not removed from the brains during this 10 day period, and the rats were perfused only on the 3 test days. Basal 5-HT values were 1.1, 1.1 and 0.5 pg/5 ul/30 min on days 1, 5, and 10 respectively; at 60 min post-injection, the 5=HT values were 11.2, 8.7, and 4.1 pg/5 ul/30 min on days 1, 5, and 10. Because chronic fenfluramine is known to deplete 5HT tissue levels (Kleven and Seiden, 1989) the decreased serotonin response in this study may be due to a mild toxic effect of the 3 - 4.0 mg/kg injections. It is more likely however, that the probe has become less viable over time. We are currently investigating these possible interpretations. This work was supported by MH-11191 and RSA-10562 (L.Seiden).

426.12

INCREASE IN TRYPTOPHAN HYDROXYLASE CONCENTRATION IN ADULT RAT NEOSTRIATUM AFTER NEONATAL DOPAMINE DENERVATION WITH 6-HYDROXYDOPAMINE. D. Weissmann*, C. Rousset*, L. Descarries and J.F. Pujol*. CNRS, UMR 105, Université Claude Bernard, Lyon, FRANCE, and CRSN (Département de physiologie), Université de Montréal, Montréal, CANADA H3C 3J7.

Using a recently developed radio-immunological technique for the visualization and measurement of enzymatic protein transferred from unfixed brain sections onto nitro-cellulose (Weissmann, D. et al., *J. Neurochem.*, 53:793, 1989), we have examined the tryptophan hydroxylase (TpOH) content in adult rat neostriatum, globus pallidus and raphe nuclei, after bilateral i.c.v. administration of 6-OHDA to 3-day-old rats pretreated with desipramine. This treatment is known to result in an almost complete dopamine denervation of the forebrain and a prominent serotonin (5-HT) hyperinnervation in the rostral half of neostriatum (Snyder, A.M. et al., *J. Comp. Neurol.*, 245:274, 1986). Three to four months after the treatment, a significant increase in tissue TpOH concentration (+ 26-36%) could be measured in the rostral but not the caudal part of neostriatum. However, there were no changes in TpOH tissue concentration in the globus pallidus nor in the nuclei raphe dorsalis, medianus or pontis. These data strongly suggest that the augmented TpOH concentration in the 5-HT-hyperinnervated neostriatum was not associated with an increased concentration of the enzyme in the nerve cell bodies of origin. If the turnover rate of TpOH is not reduced in the neostriatum, a greater amount of the protein must be produced and delivered to an enlarged axonal tree, thus maintaining the steady state concentration of the enzyme within the 5-HT nerve cell bodies.

426.13

CORRELATIVE SCANNING AND TRANSMISSION ELECTRON MICROSCOPY OF THE SUPRAEPENDYMAL NEURONAL COMPLEX IN THE HAMSTER THIRD VENTRICLE FOLLOWING RADIOFREQUENCY LESIONS OF THE MIDBRAIN RAPHE NUCLEI. R.D. Fessler*, J.C. Hazlett and J.A. Mitchell. Departments of Anatomy & Cell Biology and Neurosurgery, Wayne State University School of Medicine, Detroit, MI 48201.

Scanning electron microscopy (SEM) of the hamster (Mesocricetus auratus) third ventricle following radiofrequency lesions of the midbrain raphe nuclei (RN) reveals degeneration of the supraependymal neuronal complex (SENC) within 2-4 days (Fessler & Mitchell, Soc. Neurosci. 15: 419). The current study utilizes correlative transmission electron microscopy (TEM) to ascertain specifically which components of the SENC undergo degeneration following lesions of the RN. Hamster third ventricles previously processed for SEM were prepared for examination by TEM. TEM revealed widespread evidence of ultrastructural disruption within the core of the SENC, within the peripherally located neuronal perikarya and in supraependymal processes coursing over the floor of the third ventricle. Degenerating cell profiles and synapses exhibited increased electron density and osmophilic inclusions. In addition, increased numbers of active phagocytic profiles were noted. Incomplete lesions of the midbrain RN resulted in varying degrees of SENC perikarya and fiber degeneration. Complete lesions of the midbrain RN resulted in total loss of the SENC within 2-4 days. Control animals lesioned 1-2 mm caudal to the midbrain RN did not exhibit evidence of SENC degeneration. The results of correlative SEM/TEM following raphe lesions confirm previous SEM documentation of degeneration and suggest that the hamster SENC is dependent upon serotonergic input from the midbrain raphe nuclei.

426.14

NEUROTOXIC EFFECTS OF NEONATAL 5,7-DIHYDROXYTRYPTAMINE (5,7-DHT) TREATMENT ON RAT BRAIN TRYPTOPHAN HYDROXYLASE (TPH) AND AROMATIC-L-AMINO ACID DECARBOXYLASE (AADC). D.H. Park, T. Wessel, K.S. Kim, H. Baker, A. Towle and T.H. Joh. Lab. Mol. Neurobiol., Cornell Univ. Med. College, The Burke Rehab. Center, White Plains, NY 10605.

Recent studies indicate that in neonates the neurotoxin 5,7-DHT induces selective damage in serotonergic neurons and terminals. In the present study, we examined the effect of intracisternally applied 5,7-DHT (50 μg/pup) on the serotonin biosynthetic enzymes, TPH and AADC, using biochemical, immunocytochemical and *in situ* hybridization techniques. Enzymatic activity of TPH and AADC was measured in the dorsal raphe nucleus (DRN), caudal brainstem (CBS) and hypothalamus (HYP). One, two and four weeks after drug administration, TPH activity in all regions was reduced to 20-30% of control levels. In contrast, at one week AADC activity in DRN and CBS was decreased to 50% of control, whereas in the HYP activity increased to 125% of control. A modest decrease in AADC activity was observed in the HYP at 2 and 4 weeks after treatment. Immunohistochemical staining using serotonin and AADC antisera as well as *in situ* hybridization with a [^{35}S]-labeled TPH cDNA probe were employed to monitor the specific subnuclei of the DRN exhibiting the biochemical alterations. Up to four weeks after drug treatment, many neurons could be demonstrated in medial aspects of the DRN. However, the neurons were absent from the lateral subnuclei of DRN and the B9 group. At higher doses of 5,7-DHT (200 μg/pup) neuronal loss in the DRN was more widespread. The relatively large decrease in TPH activity as compared to neuronal loss produced at low doses of 5,7-DHT suggests that enzyme synthesis may be compromised even in the remaining neurons. These data demonstrate a dose-dependent selective vulnerability of midbrain serotonergic neurons to 5,7-DHT.

426.15

EFFECTS OF RAPHE HYPERINNERVATION ON STRIATAL SEROTONERGIC NEUROTRANSMISSION IN RATS DEPLETED OF DOPAMINE AS NEONATES. D. Jackson, M. Teitler*, S. Leonhardt*, M.J. Zigmond, and E.D. Abercrombie, *Dept. of Behavioral Neuroscience, Univ. of Pittsburgh, Pittsburgh, PA., *Dept. Pharm. & Tox., Albany Med. Col., Albany, N.Y.*

Neonatal rats treated with 6-hydroxydopamine (6-HDA) fail to show the profound behavioral deficits observed in rats receiving comparable lesions as adults. This sparing is accompanied by extensive sprouting of serotonin (5-HT) neurons in striatum. However, behavioral and biochemical studies suggest that 5-HT function actually is reduced in neonatally-lesioned rats. Thus, 5-HT, which normally inhibits acetylcholine release, fails to do so in these animals (Jackson et al., Brain Res., 457: 267, 1988). We are exploring two explanations: that 5-HT is not released or that sensitivity to 5-HT is lost in neonatally-lesioned rats. Using microdialysis we monitored extracellular 5-HT in response to fluoxetine, an uptake blocker (100 uM) or KCl (100 uM) via the probe, or fenfluramine (10 mg/kg, i.p.), a 5-HT releasing agent:

Extracellular 5-HT (pg/50 ul dialysate)

Condition (n)	Control	Lesion	% Control
Basal (12)	12.5 ± 1	9.4 ± .8	75
Fluoxetine (4)	200 ± 9	689 ± 200	345
KCl (4)	201 ± 56	578 ± 115	288
Fenfluramine (4)	193 ± 41	554 ± 143	287

The response to each treatment was greater in the lesioned animals. However, the apparent increase in the capacity for 5-HT release may be offset by an increase in 5-HT uptake sites, thus obviating any advantage conferred by 5-HT sprouting. Preliminary results indicate that 5-HT_2 receptor binding was not altered in lesioned neonates. Thus, both 5-HT release and 5-HT_2 receptor sites appear intact after 6-HDA-induced hyperinnervation of 5-HT afferents to striatum.

5HT_{1a} AND OTHER 5HT RECEPTOR SUBTYPES

427.1

5-HT_{1A} AGONIST-LIKE EFFECTS OF NAN-190. J.T.Lum, W.E.Hoffmann, and M.F. Piercey, The Upjohn Company, Kalamazoo, MI 49001.

Like many other 5-HT_{1A} agonists (e.g. buspirone, ipsapirone), NAN-190 is an arylpiperazine which binds to 5-HT_{1A} receptors. However, unlike 5-HT_{1A} agonists, NAN-190 antagonized the discriminative effects of 8-OH DPAT (Glennon *et al.*, Drug Dev. Res. 16: 335-343, 1989). We have now further evaluated NAN-190's effects on bioassays sensitive to 5-HT_{1A} agonists. In hypothermia assays, mice were treated with 10 mg/kg 8-OH DPAT to give a temperature drop of 6.0±0.9°F. NAN-190 (gift of R.A.Glennon) failed to antagonize this effect of 8-OH DPAT, but did itself depress body temperature. To more specifically evaluate on 5-HT mechanisms, we measured its effects on 5-HT neuron firing rates. Dorsal raphe 5-HT neurons identified according to Aghajanian *et al.*, (JPET 137:178, 1970) were depressed by 8-OH DPAT (ED_{50} = 1.6 µg/kg). Again, NAN-190 did not reverse the depressant effects of 8-OH DPAT, but did itself depress firing rates with a potency (ED_{50} = 11 µg/kg) similar to those for buspirone, ipsapirone, and gepirone (7-15 µg/kg). It is concluded that behavioral antagonism by NAN-190 is unlikely to result from blockade of presynaptic 5-HT_{1A} receptors, but could result from antagonism of postsynaptic 5-HT_{1A} receptors.

427.2

INHIBITION OF DORSAL RAPHE CELL FIRING BY MDL 73005EF: RELATIONSHIP TO 5-HT_{1A} RECEPTOR BINDING. J.S. Sprouse, D.R. McCarty* and M.W. Dudley, Merrell Dow Research Institute, 2110 E. Galbraith Road, Cincinnati, OH 45215

MDL 73005EF (8-[2-(2,3-dihydro-1,4-benzodioxin-2-ylmethylamino)-ethyl]-8-azaspiro[4,5]decane-7,9-dione methylsulphonate) has been shown to be an effective agent in preclinical assessments of anxiolytic drug action. The goal of the present study was to determine whether MDL 73005EF is an agonist at the 5-HT cell body autoreceptor (5-HT_{1A}) in the dorsal raphe (DR) and whether this action is consistent with binding to the autoreceptor in DR membranes. Extracellular single unit recordings of DR neurons were made in the in vitro rat brain slice. MDL 73005EF potently inhibited neuronal firing in a concentration-dependent manner (IC_{50} = 129 ± 34 nM; N=6), typical of the non-indole 5-HT_{1A} agonists. In bovine DR membranes, MDL 73005EF effectively displaced [^{3}H] 8-OH-DPAT binding; however, the data could be resolved into a 2-site model with a low (IC_{50} = 2.57 ± 0.64 µM) as well as a high (4.38 ± 1.30 nM) affinity binding site (Hill coefficient = 0.38). Of the total labeled sites, ~60% were low affinity sites. A similar pattern emerged when 5-HT was used as the displacing agent (1.17 ± 0.25 µM, 4.00 ± 1.40 nM). In contrast, in membranes prepared from bovine hippocampi (HC), also rich in 5-HT_{1A} sites, displacement of [^{3}H] 8-OH-DPAT binding by MDL 73005EF and 5-HT revealed only the high affinity site (3.37 ± 0.56 nM and 5.81 ± 0.61 nM, respectively). The functional role for these "states" of the 5-HT_{1A} receptor is unknown, but the differences observed in their relative proportion in the DR and HC may explain in part the agonist or partial agonist profiles of 5-HT_{1A} ligands at these receptor sites.

427.3

IN VIVO PERTUSSIS TOXIN (PT) EFFECTS ON 5-HT_{1A} AND D2 AUTO-RECEPTOR FUNCTIONAL RESPONSE: POSSIBLE RELATIONSHIP TO RECEPTOR RESERVE (RR). K. Bohmaker and E. Meller. Dept. of Psychiatry, NYU Medical Center, New York, New York 10016.

We previously demonstrated the presence of a large RR for agonists at both D2 and 5-HT_{1A} autoreceptors. Since both of these receptors appear to be coupled to their effectors via PT-sensitive G-proteins, it was of interest to determine the effects of PT treatment on the pharmacological response to full and partial D2 and 5-HT_{1A} agonists. Rats were treated intrastriatally or in the vicinity of the dorsal raphe nucleus with 0.5-1.0 ug of PT or vehicle. After 48 hr, dose-response (DR) curves were obtained for full and partial agonists at the receptor of interest (D2:(-)NPA and (+)3-PPP; 5-HT_{1A}: 8-OH-DPAT and buspirone). D2 and 5-HT_{1A} autoreceptor function were assessed as agonist-induced reversal of L-DOPA accumulation after GBL and inhibition of 5-HT synthesis, respectively. Analogous to our previous findings after irreversible receptor inactivation, PT treatment shifted the DR curves for full agonists to the right, with little or no reduction in maximal response, whereas for partial agonists the maximal response was reduced with little or no shift in ED_{50}. These results suggest that RR may be related to a "G-protein reserve" for full but not partial agonists. Even for a full agonist, no "G-protein reserve" would be predicted in the absence of RR; this is indeed the case for 5-HT_{1A} mediated inhibition of forskolin-stimulated adenylate cyclase (see Yocca et al., this meeting). Supported by NINDS grant NS-23618.

427.4

LACK OF RECEPTOR RESERVE (RR) AT POSTSYNAPTIC 5-HT1A RECEPTORS LINKED TO ADENYLYL CYCLASE (AC) IN RAT HIPPOCAMPUS (HIP). F.D. Yocca[1], L. Iben*[1] and E. Meller[2] CNS Research Bristol-Myers Squibb Co., Wallingford, CT 06492[1] and Dept. of Psychiatry, NYU Medical Center, NY, NY 10016.[2]

Multidisciplinary studies have shown that 5-HT_{1A} agonists appear to exhibit higher efficacy at somatodendritic vs postsynaptic 5-HT_{1A} receptors. A possible explanation for this phenomena is the existence of a RR in the dorsal raphe (DR). This reserve may result from an excess of either the 5-HT_{1A} receptor or the G-protein linked to this receptor. To test this hypothesis we compared the 5-HT_{1A} receptor system in the rat HIP to that in the DR. Through receptor alkylation via treatment with N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ), presynaptic DR 5-HT synthesis-sensitive 5-HT_{1A} autoreceptors in rat were shown to possess a large receptor reserve for 8-OH-DPAT (Meller et al., Mol. Pharm., 37:231-237, 1990). Furthermore, ADP ribosylation of G_i-protein via infusion of pertussis toxin (PT) into rat DR results in a dextral shift of the EC_{50} for 8-OH-DPAT with little or no change in the E_{max}, suggesting the exsistence of a "G-protein reserve" (see Bohmaker and Meller, this meeting). Similar techniques were used here to determine the reserve status of rat postsynaptic HIP 5-HT_{1A} receptors linked negatively to AC. Irreversible receptor alkylation by EEDQ reduced the maximal response but did not alter the EC_{50} for 5-HT. Similarly, PT infusion into rat HIP reduced the maximal response of 5-HT with little or no change in EC_{50} (Clarke et al., Brain Res., 14:361-367, 1987). These results indicate that postsynaptic 5-HT_{1A} receptors, as opposed to somatodendritic autoreceptors, do not possess a RR, which may in part explain the differences in relative efficacies demonstrated by 5-HT_{1A} agonists at these sites. Furthermore, these findings are consistent with the idea that receptor reserve may be related to a "G-protein reserve".

427.5

REGIONAL INTERACTIONS OF 8-OHDPAT WITH p-Cl-AMPH AND MDMA IN VIVO IN RAT BRAIN. J. E. Embury, A. Frank*, and M. P. Galloway. Dept. Psychiatry, Cellular & Clinical Neurobiology Program, Wayne State Univ Sch Med, and Lafayette Clinic, Detroit MI

Using cortical slices in vitro, we have previously reported that both the 5-HT 1A agonist 8-OHDPAT, and 5-HT reuptake inhibitors block the 5-HT releasing effects of either 5-HT 1 agonists (eg, TFMPP, RU 24969), or 5-HT toxins (eg, p-Cl-AMPH, MDMA) thus providing evidence for a functional interaction between 5-HT-1 receptor agonists with the 5-HT reuptake site (Galloway et al., this meeting). The present study examined potential interactions between 5-HT-1 receptor agonists and the 5-HT reuptake site in vivo. Fluoxetine (5 mg/kg, s.c.), a 5-HT reuptake inhibitor,completely blocked the ability of p-Cl-AMPH (5 mg/kg s.c.), to reduce endogenous 5-HT levels in both the medial prefrontal cortex (MPF) and the striatum (STR). Pretreatment with 8-OHDPAT at 0.5 and 1.0 mg/kg resulted in a 46% and 70% reversal of the p-Cl-AMPH depletion of striatal 5-HT, respectively. (+)-MDMA (10 mg/kg, s.c., administered at 1.0, 1.5, 2.0 and 3.0 hrs. before sac.) inhibited 5-HT synthesis in the anterior and posterior STR, MPF, nucleus accumbens (NAc), HC, and temporal cortex and decreased 5HT levels (10 mg/kg, s.c., 3 hrs. before sac.) in the STR, MPF and HC. In contrast, MDMA did not decrease 5-HT in the NAc or OT and actually increased 5-HT synthesis in the OT. The 5-HT depleting effect of MDMA in the MPF was partially reversed by pretreatment with 8-OHDPAT. Nomifensine (10 mg/kg, s.c.), a dopamine reuptake inhibitor, blocked the ability of 8-OHDPAT to reverse MDMA toxicity in the MPF. The present study suggests an interaction between 8-OHDPAT and the 5-HT reuptake site in vivo with a possible dopaminergic influence that is specific for DA projection fields. (Supported by MH-41227, DA-04120, and the State of Michigan DMH).

427.6

8-OHDPAT, LIKE 5HT REUPTAKE INHIBITORS, BLOCKS THE EFFECTS OF MDMA AND TFMPP ON 5HT RELEASE AND SYNTHESIS IN VITRO. M.P. Galloway, E.A.Novak*, B.N.Mathews* Lafayette Clinic, Cellular & Clinical Neurobiol. Prog., Dept. Psychiatry, Wayne State Univ Sch Med, Detroit MI

We have previously reported that 5HT agonists such as TFMPP, m-CPP, and RU 249696 stimulate endogenous 5HT release from cortical slices, an effect which is blocked the 5HT-1A agonist 8-OHDPAT (which alone has no effect on 5HT release). Our results suggested that the releasing agents were substrates for the 5HT transporter whereas 8-OHDPAT acted as a reuptake inhibitor. Based on this premise, we recently found that either 8-OHDPAT, sertraline, or fluoxetine dose-dependently blocks the in vitro 5HT releasing properties (from cortical slices) of the 5HT neurotoxins (+)MDMA ("ecstasy") and p-Cl-AMPH. Ipsapirone, a chemically dissimilar 5HT-1A agonist, only weakly reversed MDMA, whereas other aminotetralins (7-OHDPAT > 5-OHDPAT > AJ-76) significantly reduced the 5HT releasing action of MDMA. 8-OHDPAT was more effective on MDMA induced release of striatal 5HT, when compared to striatal DA release. Further evidence that TFMPP is a substrate for the 5HT transporter was the observation that a 50% reduction in $[Na^+]_{EXT}$ in the media blocked the release of 5HT by TFMPP. However, removal of Ca^{++} blocked neither TFMPP induced 5HT release nor the antagonism afforded by 8-OHDPAT. These results demonstrate a functional interaction of 5HT agonists with the 5HT transporter: 8-OHDPAT resembles classical 5HT reuptake inhibitors in that both block the 5HT releasing properties of TFMPP, RU 24969, MDMA, or p-CA, compounds thought to be substrates at the 5HT transporter. Support: MH-41227 DA-04120 State of Michigan, Department of Mental Health.

427.7

CP-93,129, A NEW SEROTONERGIC LIGAND WITH MARKED AFFINITY AND HIGH SELECTIVITY FOR 5-HT$_{1B}$ RECEPTORS. B.K. Koe, L.A. Lebel, C.A. Burkhart*, and J.E. Macor*. Central Research Division, Pfizer Inc., Groton, CT 06340

The mechanism of action of several new classes of therapeutic drugs involves modulation of central serotonergic function. These include antidepressants based on selective blockade of serotonin (5-HT) uptake (fluoxetine, sertraline), new anxiolytics based on 5-HT$_{1A}$ agonist activity (buspirone, tandospirone) and an anorectic agent based on enhancing 5-HT release (dexfenfluramine). The recent description of new 5-HT receptors and receptor subtypes affords the opportunity of designing novel ligands that will exert a selective action at a targeted receptor subtype. Our efforts directed at the 5-HT$_{1B}$ receptor, apparently the major 5-HT$_1$ subtype in rat brain other than 5-HT$_{1A}$, led to the synthesis of CP-93,129, the tautomer of 5-hydroxy-3-(4-1,2,5,6-tetrahydropyridyl)-4-azaindole. CP-93,129 was found to exhibit marked affinity for 5-HT$_{1B}$ receptors in rat brain membranes (IC_{50} 15 nM using [^{3}H]5-HT). The high selectivity of CP-93,129 for 5-HT$_{1B}$ receptors (rat cortex) was shown by the following binding results: 200-fold greater affinity for 5-HT$_{1B}$ than 5-HT$_{1A}$ sites (rat cortex), 150-fold greater affinity for 5-HT$_{1B}$ than 5-HT$_{1D}$ sites (bovine striatum), 400-fold greater affinity for 5-HT$_{1B}$ than 5-HT$_{1C}$ sites (pig choroid plexus) and 2500-fold greater affinity for 5-HT$_{1B}$ than 5-HT$_2$ sites (rat cortex). CP-93,129 showed virtually no affinity for noradrenergic or dopamine receptors and did not block 5-HT uptake or bind to the 5-HT uptake site. An agonist function for CP-93,129 was indicated by a decrease in binding affinity in the presence of Gpp(NH)p. CP-93,129 may be a useful agent for ascertaining the pharmacological and behavioral role of 5-HT$_{1B}$ receptors.

427.8

CP-93129: A POTENT AND SELECTIVE 5-HT$_{1B}$ RECEPTOR AGONIST. A.W. Schmidt, J.E. Macor*, and D.W. Schulz. Central Research Division, Pfizer Inc., Groton, CT 06340.

Highly selective ligands, such as 8-OH DPAT, have contributed greatly to our understanding of the functional properties of the 5-HT$_{1A}$ receptor. Comparable studies of 5-HT$_{1B}$ receptors have been hampered in part by a lack of compounds having high 5-HT$_{1B}$ receptor selectivity. However, it was recently established that CP-93129 [3-(1,2,3,6-tetrahydropyrid-4-yl)-pyrrolo[3,2-b]pyrid-5-one] has >200-fold selectivity for 5-HT$_{1B}$ vs 5-HT$_{1A}$ sites in rat brain, while binding to 5-HT$_{1C}$ and 5-HT$_2$ receptors with affinities in the micromolar range (Koe *et al.*, Soc. Neurosci., 1990).

CP-93129 competed for ^{3}H-5HT binding to 5-HT$_{1B}$ receptors in rat cortex with a K_i of <5 nM, but inhibited ^{3}H-8-OH-DPAT binding in the same tissue with a K_i of >1 μM. CP-93129 behaved as an agonist in inhibiting forskolin-stimulated adenylate cyclase activity in guinea pig hippocampus (5-HT$_{1A}$ receptors), rat substantia nigra (5-HT$_{1B}$ receptors), and guinea pig substantia nigra (5-HT$_{1D}$ receptors), with EC_{50} values in these three preparations of >10 μM, 56 nM, and >10 μM, respectively. CP-93129 also potently inhibited forskolin-stimulated adenylate cyclase in rat globus pallidus, an area rich in 5-HT$_{1B}$ receptors. The K^+-induced release of endogenous 5-HT was significantly inhibited by CP-93129 in globus pallidus synaptosomes. Thus, CP-93129 represents a novel tool for investigating both pre- and postsynaptic 5-HT$_{1B}$ receptor function.

427.9

CP-93,129, A POTENT AND SELECTIVE 5HT$_{1B}$ AGONIST, INHIBITS FOOD INTAKE IN RATS. L.K. Torgersen*, J.E. Macor* and J.A. Nielsen. Central Research Division, Pfizer Inc., Groton, CT 06340.

Serotonin (5HT) is involved in the regulation of food intake. Direct infusion of 5HT into the paraventricular nucleus of the hypothalamus (PVN) of rats leads to inhibition of feeding with less than full efficacy. This partial effect may result from activation of 5HT$_{1B}$ receptors which produce anorexia plus 5HT$_{1A}$ receptors which cause hyperphagia. CP-93,129 is a potent (IC_{50}= 15 nM) and selective (150-200 fold greater affinity than for the 5HT$_{1A}$ or 5HT$_{1D}$ sites) 5HT$_{1B}$ ligand. CP-93,129 inhibits forskolin-stimulated adenylate cyclase, suggesting agonist activity at the 5HT$_{1B}$ receptor. The present investigation studies the effects of CP-93,129 on food intake in rats.

CP-93,129 inhibited food intake when infused into the PVN, an effect that was both dose-related (ED_{50} = 8 μg) and time-dependent (30 min duration of anorexia at ED_{50}). The 5HT$_{1B}$ partial agonists metergoline and methysergide partially prevented CP-93,129 induced anorexia when they were injected either ip or directly into the PVN. CP-93,129 had no effect on food intake when injected systemically, perhaps because its structure does not allow free distribution to the brain. CP-93,129, injected into the PVN, was a more efficacious anorexic than 5HT. 5HT might be a less efficacious anorexic than CP-93,129 due to its 5HT$_{1A}$ agonist activity.

These data suggest that central 5HT$_{1B}$ receptors modulate food intake in the rat.

427.10

[^{3}H]CP-96,501, A NEW RADIOLIGAND FOR 5-HT$_{1B}$ RECEPTORS IN RAT BRAIN. L.A. Lebel, C.A. Burkhart*, J.E. Macor*, and B.K. Koe. Central Research Division, Pfizer Inc., Groton, CT 06340

The recent description of different 5-HT$_1$ receptor subtypes in brain has prompted interest in discovering selective agonists and antagonists in order to ascertain the role of the various sites in serotonergic function. The major 5-HT$_1$ subtype, other than 5-HT$_{1A}$, in rat brain is 5-HT$_{1B}$. Standard 5-HT$_{1B}$ agonists used in the literature include RU 24969 (5-MeO-3-(4-1,2,5,6-tetrahydropyridyl)indole), MCPP, TFMPP and CGS 12066B. Of these, RU 24969 exhibits the greatest affinity for 5-HT$_{1B}$ receptors (IC_{50} 1.9 nM) with a 7-fold selectivity over 5-HT$_{1A}$ sites. The 5-n-PrO analog of RU 24969 (CP-96,501) was synthesized and found to be equally potent at 5-HT$_{1B}$ receptors with a 40-fold selectivity over 5-HT$_{1A}$ sites. [^{3}H]CP-96,501 was synthesized and evaluated as a 5-HT$_{1B}$ radioligand. Binding experiments were conducted with whole rat brain membranes in the absence of any binding "masks." [^{3}H]CP-96,501 was found to display high affinity binding: K_D 0.16 nM; B_{max} 68 fmol/mg protein; Hill coefficient 0.98. The K_D calculated from on- and off-rates was in good agreement (0.14 nM). Rosenthal plots of [^{3}H]CP-96,501 binding with added 5-HT (5 nM) or TFMPP (20 nM) indicated competitive inhibition. The potency of various serotonergic compounds (e.g., 8-OH-DPAT, CGS 12066B, GR 43175, mesulergine) from competition curves in [^{3}H]CP-96,501 binding showed good correlation (r = 0.99) with their inhibitory activity in [^{3}H]5-HT binding to 5-HT$_{1B}$ receptors. An agonist function for CP-96,501 was suggested by the decrease in binding affinity in the presence of Gpp(NH)p. Our findings indicate that [^{3}H]CP-96,501 is a specific agonist ligand for 5-HT$_{1B}$ receptors in rat brain. [^{3}H]CP-96,501 saturation binding in brain membranes from several other species did not yield definitive evidence to support the presence of 5-HT$_{1B}$ receptors in these tissues.

427.11

DEVELOPMENT OF SELECTIVE/POTENT 5-HT1A ANTAGONISTS. R. K. RAGHUPATHI[a], L. RYDELEK-FITZGERALD[#], M. TEITLER[#], R. A. GLENNON[a]*. [a]Dept. of Med. Chem., MCV/VCU, Richmond, VA 23298, and [#]Dept. of Pharmacol. and Toxicol., Albany Medical College, Albany, NY 12208.

NAN-190, 1-(2-methoxyphenyl)-4-[4-(2-phthalimido)butyl]piperazine, has been previously reported by our laboratory as a high-affinity (Ki = 0.6 nM) 5-HT1A antagonist. (Glennon et al., Eur. J. Pharmacol. 154:339, 1988). We attempted to overcome the problem of its equal affinity (Ki = 0.8 nM) for α1-adrenergic sites by performing structure-affinity relationship (SAFIR) studies for binding at each of the two sites. Replacement of the aromatic phthalimido moiety of NAN-190 by alkyl amide groups decreases α1 affinity with retention of high affinity for 5-HT1A sites. Increased branching and bulkiness of the alkyl amide portion shows a trend towards increased 5-HT1A selectivity. We have thus been able to obtain compounds with a >100-fold selectivity for [^{3}H]8-OH DPAT-labelled 5-HT1A sites over α1-adrenergic sites. These compounds (e.g. RK-153, Ki = 0.4 nM) show antagonist activity in the 5-HT1A-coupled adenylate cyclase assay. We have therefore succeeded in developing several novel high-affinity 5-HT1A ligands that lack as significant an α1 component as NAN-190. (Supported in part by PHS grant NS 23523).

427.12

SEROTONIN-1$_A$ AND SEROTONIN-2 RECEPTORS IN EUTHERMIC AND TORPOR PRONE DEERMICE, PEROMYSCUS MANICULATUS. B.A. Hulihan-Giblin, E.B. Pivorun*, D. Goldman NIAAA, Section on Genetic Studies, LCS Bethesda, Maryland 20892.

P. maniculatus, the deermouse, displays both spontaneous and induced daily torpor bouts, attaining minimum body T of 15-20 C. There is evidence that brain serotonin may be involved in the initiation and/or maintenance of torpor. Inhibition of serotonin synthesis markedly reduces the duration and depth of torpor (Pivorun and Astwood, Living in the cold, pp. 323-29, 1986) while an increase in activity of the serotonergic and dopaminergic systems has been shown in the hypothalamus and SCN during daily torpor (Lin and Pivorun, Pharm Biochem and Behav. 33:309-14, 1989). A certain percentage of deermice will not enter torpor under any circumstances (30-40%). We compared 5-HT receptor subtypes in deermice that readily enter into torpor (TP) and in non-torpor prone (NTP) animals. Deermice were trapped in the wild and subjected to food rationing and low ambient T and then sacrificed in a normothermic or torpid state at 11 P.M. or at 11 A.M. Whole brain was assayed for 5HT1$_A$ and 5HT2 receptor differences using [^{3}H]8-OH-DPAT and [^{3}H]ketanserin, respectively. Serotonin-1$_A$ brain receptors revealed both diurnal and genetic functional differences. The density (Bmax) of 5HT1$_A$ brain receptors was higher in both TP and NTP animals at 11 P.M. then at 11 A.M. Furthermore, at 11 P.M., 5HT1$_A$ receptor binding was significantly higher in TP animals compared to the NTP. No significant difference was seen at 11 A.M. Serotonin-2 receptors displayed the same diurnal variation as 5HT1$_A$ receptors. However, 5HT2 brain receptors were higher in NTP animals as compared to TP animals sacrificed at 11 A.M. These diurnal and genetic receptor differences in TP and NTP animals indicate that certain serotonin receptors may be involved in the modulation of torpor in this species.

427.13

5-HT RECEPTOR SUBTYPES IN RAT DORSAL ROOT GANGLION (DRG) CELLS AND THEIR ASSOCIATED IONIC CONDUCTANCES. Slobodan Todorovic and E.G. Anderson Dept. Pharmacology, U of I Chicago, Chicago, IL 60612.

Rat DRG neurons exhibit 3 distinct membrane responses to 5-HT; depolarization with increased or decreased input resistance (Rin) and hyperpolarization. Depolarization with increased Rin is mediated by 5-HT2 receptors while depolarization with decreased Rin is mediated by 5-HT3 receptors (Brain Research, 511, 71-79, 1990). We have now estimated reversal potentials (rP) from current-voltage curves constructed in the presence and absence of 5-HT. In cells depolarized by 10 μM 5-HT with increased Rin the rP was -100.75±1.8 mV (N=4) in 2.5 mM K+, and the rP for the 5-HT2 agonist alpha-Methyl 5-HT (10μM) was -97±3.9 mV (N=5). Increases in the extracellular K+ produced a linear shift in the rP as predicted by the Nernst equation, indicating the 5-HT2 response is mediated by a decrease in K+ conductance. In cells depolarized by 1 μM 5-HT with decreased Rin, the rP was -30.2 ± 1.8 mV (N=5) and with the selective 5-HT3 agonist 2-methyl 5-HT (1 μM) the rP was -32 mV (N=2). K+ acetate electrodes did not alter this response. In Na+ free solution, both agonists produced hyperpolarization, indicating this response involves increased Na+ conductance. The negative rP suggests that an increased K+ conductance also occurs.

In 18 cells 5-HT produced a hyperpolarization with decreased Rin. One μM 5-HT achieved an Emax averaging -4.05±0.6 mV (N=9). This was mimicked by the 5-HT1 agonist, carboxamidotryptamine (Emax = 4.2±0.7 mV, 1 μM, N=5). This response was not blocked by ketanserin or ICS 205-930, but 100nM methiothepin (a broad 5-HT1/5-HT2 antagonist) produced a parallel rightward shift in the 5-HT cocentration response curve. These data suggest that a 5-HT1 receptor mediates hyperpolarization in rat DRG cells.

427.14

THE BIOCHEMICAL AND FUNCTIONAL CORRELATE OF 5-HT$_2$ RECEPTOR ACTIVATION IN IMMATURE AND ADULT RAT BRAIN CORTEX. J. Coupet and P. Tricoli*, Med. Res. Div. of American Cyanamid Company, Ramapo Coll, Mahwah, NJ 07430.

Representatives of a class of hallucinogenic -4-substituted phenylalkylamine: 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane (DOM), 1-(2,5-dimethoxy-4-bromophenyl)-2-aminopropane (DOB) and 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI), characterized in ligand binding assays as 5-HT$_2$ selective agonists, were investigated in polyphosphoinositide (PPI) hydrolysis, the transducing system for 5-HT$_2$ receptors, in immature as well as adult rat cortical slices. Inositol phospholipids in cortical slices were prelabeled with myo-2-[^{3}H]inositol and the formation of total [^{3}H]inositol phosphates was measured in the presence of 7mM LiCl. The PPI response to these 5-HT$_2$ agonists was considerably more pronounced in the immature than in the adult animals. The involvement of the 5-HT$_2$ receptor in modulating the PPI response was strongly supported, on one hand, by the inhibitory effect of ketanserin, a 5-HT$_2$ antagonist, and on the other hand, by the failure of CGS-12066B and 1-phenylbiguanide, a 5-HT$_{1B}$ and a 5-HT$_3$ agonist, respectively, to affect the formation of [^{3}H]IP. It was concluded that the hyperresponsiveness of PPI hydrolysis to the various 5-HT$_2$ agonists in the immature rat brain could be a compensatory mechanism due perhaps to a tighter coupling state of the G-protein to the 5-HT$_2$ receptors, at an early stage of brain development.

427.15

Serotonin (5-HT) excites interneurons via 5-HT$_2$ receptors and pyramidal cells via 5-HT$_{1C}$ receptors in rat piriform cortex. P.W. Sheldon and G.K. Aghajanian, Depts. of Pharmacology and Psychiatry, Yale University, New Haven CT. 06510.

We have shown previously that serotonin (5-HT) both depolarizes pyramidal cells and induces firing of a subpopulation of interneurons in the piriform cortex. The 5-HT$_{2/1C}$ antagonist ritanserin blocked the activation of interneurons more readily than it blocked the depolarization of pyramidal cells. Because ritanserin has a nearly 10 fold higher affinity for the 5-HT$_2$ receptor than for the 5-HT$_{1C}$ receptor (Hoyer. 1988, J. Receptor Res. 8(1-4): 59-81) it was hypothesised that the action of 5-HT on these interneurons might be through 5-HT$_2$ receptors and the action of 5-HT on the pyramidal cells might be through 5-HT$_{1C}$ receptors. This hypothesis is consistent with recent findings of Palacios (personal communication) that the mRNA for the 5-HT$_2$ receptor is found in cortical interneurons while mRNA for the 5-HT$_{1C}$ receptor is found in pyramidal cells.

To test this hypothesis further we examined the effects of the 5-HT antagonist spiperone which has a 1000 fold higher affinity for the 5-HT$_2$ receptor than for the 5-HT$_{1C}$ receptor (Hoyer). In an *in vitro* slice preparation of rat piriform cortex we studied the actions of 5-HT on both interneurons and pyramidal cells. The activating effect of 5-HT on interneurons was blocked by bath-application of spiperone (100nM). 5-HT increased the excitability of pyramidal cells as measured by its ability to increase the number of spikes evoked by a constant depolarizing current pulse; this effect of 5-HT was uneffected by 100nM spiperone but was blocked by 10μM ritanserin. These findings indicate that in the piriform cortex the excitatory action of 5-HT on interneurons is mediated by 5-HT$_2$ receptors while the excitatory action of 5-HT on pyramidal cells is mediated by 5-HT$_{1C}$ receptors.
Supported by PHS Grants GM07324 and MH17871.

427.16

DESTABILIZATION OF HORMONE / RECEPTOR / G-PROTEIN (H-R-G) TERNARY COMPLEX IS DEPENDENT ON RECEPTOR OCCUPANCY BUT INDEPENDENT OF DRUG EFFICACY. C.D. Mahle[1], H.L. Wiener[3,2], F.D. Yocca[4,2], and S. Maayani[1,2]. Depts. Pharmacol.[1] & Anesthesiol.[2], Mt. Sinai Sch. Med., CUNY, NY, NY 10029, Coll. Pharmacy[3], St. John's U., Jamaica, NY 11439, and CNS Research, Bristol Myers-Squibb[4], Wallingford, CT 06492.

Destabilization of H-R-G by guanine nucleotides (GN) was utilized as a model to study initial events of signal transduction. A crucial step is the allosteric interaction between hormone receptor sites and GN G-protein sites. This phenomenon was examined in rat hippocampal membranes on two G$_i$-linked receptors, the adenosine A$_1$ (AD A$_1$), labeled by the agonist [^{3}H]-R-phenylisoproplyadenosine ([^{3}H]-R-PIA), and the 5-HT$_{1A}$ receptor labeled by either the agonist [^{3}H]-8-OH-2-(di-n-propylamino)-tetralin) ([^{3}H]-8-OH-DPAT) or the partial agonist [^{3}H]-BMY 7378. Progressive occupancy of the G-protein with either 5'guanylimidodiphosphate (Gpp(NH)p) or guanosine 5'-O-(3-thiotriphosphate) (GTPγS) resulted in a concentration-dependent and saturable attenuation of [^{3}H]-agonist binding, with GTPγS exhibiting a lower IC$_{50}$ for all drugs. Parameters of concentration-effect curves (IC$_{50}$, slope index and E$_{max}$) were determined for Gpp(NH)p and GTPγS at different occupancy levels of the receptor sites. For all drugs, IC$_{50}$ values increased with increasing receptor occupancy, while maximal inhibition of [^{3}H]-R-PIA or [^{3}H]-8-OH-DPAT binding was independent of receptor occupancy. Both GN exhibited lower IC$_{50}$ values on the 5-HT$_{1A}$ agonists than those on the AD A$_1$ agonist. Similar IC$_{50}$ values were obtained for both [^{3}H]-8-OH-DPAT and [^{3}H]-BMY 7378 binding, suggesting efficacy independence in this event of receptor/G-protein coupling. We propose that characteristics of destabilization of the H-R-G ternary complex by GN are dependent upon receptor type and degree of receptor occupancy, but are independent of drug efficacy. (USPHS GM 34852)

427.17

LACK OF 5-HT INHIBITION OF ADENYLYL CYCLASE IN DORSAL RAPHE OF MALE AND FEMALE RATS. W.P. Clarke[1], F.D. Yocca[2,3] and S. Maayani[1,2] Departments of Pharmacology[1] and Anesthesiology[2], Mount Sinai School of Medicine, CUNY, New York, NY 10029 and CNS Research[3], Bristol-Myers Squibb Co. Wallingford, CT 06492.

In rat hippocampus, the serotonin$_{1A}$ (5-HT$_{1A}$) receptor subtype is linked to at least two distinct responses: 1) inhibition of pyramidal cells, mediated by the opening of a non-cAMP dependent K^+ channel (Andrade *et al.*, Science, 234:1261, 1986) and 2) inhibition of forskolin-stimulated adenylyl cyclase (FSAC) (De Vivo and Maayani, JPET, 238:248, 1986). In ovariectomized (OVX) rats, estrogen enhances both of these 5-HT$_{1A}$ mediated responses (Beck *et al.*, Neurosci. Lett., 186:181,1989; Clarke and Maayani, Brain Res., 518:287, 1990). However, in dorsal raphe (DR), estrogen treatment of OVX rats decreases 5-HT$_{1A}$ inhibition of firing of cells (Lakoski, The Pharmacologist, 30:A126, 1988). In this study we tested the hypothesis that a lack of estrogen (in OVX rats) would enhance 5-HT$_{1A}$ mediated inhibition of FSAC activity in the DR. OVX rats each received a silastic capsule (sc) containing either 10% estradiol-17β in cholesterol (CHOL) or CHOL alone. Male rats were untreated. Four days later, membranes from DR were prepared (8 rats/group). Virtually no inhibition (<5%) of FSAC by 5-HT was seen in the DR of OXV-estrogen, OVX-CHOL or male rats, whereas in hippocampus maximal inhibition by 5-HT averaged 25%. In contrast, inhibition of FSAC in DR did occur with R-phenylisopropyl-adenosine (adenosine A_1, 20%), baclofen ($GABA_B$, 20%) and with guanosine 5'-(β,γ-imino)triphosphate (Gpp(NH)p, 40%). While conclusions related to estrogen's effect in DR are precluded due to the lack of response to 5-HT, these data suggest that there may exist subpopulations of the 5-HT$_{1A}$ receptor, linked to either a K^+ channel or to adenylyl cyclase, which have a heterogenous distribution in the CNS; the hippocampus possessing 5-HT$_{1A}$ receptors coupled to both effectors (possibly on different cells) and the raphe with 5-HT$_{1A}$ receptors linked only to a K^+ channel. Supported by grants GM-34852 and DA-01875. WPC is a Revson Fellow.

427.18

INITIAL CHARARCTERIZATION OF 5-HT RECEPTOR PRESENT IN *ASCARIS SUUM* MUSCLE. J. Williams and A. Shahkolahi*. Department of Biological Sciences, University of North Texas, Denton, TX 76203.

Ascaris suum is a parasitic nematode which inhabits the upper third of the small intestine of porcine species. During non-feeding periods of the host *A. suum* is dependent upon muscle glycogen stores. 5-hydroxytryptamine (5-HT) initiates glycogenolysis in *A. suum* muscle via an increase in cAMP levels. Functionally, the 5-HT1 receptor has been linked to the cAMP system, the 5-HT2 receptor has been linked to the phosphotidylinositol system, and the 5-HT3 receptor to ligand gated ion channels. Although ligand binding to the *A. suum* 5-HT receptor appears to initiate glycogenolysis through the cAMP system and not through the phosphotidylinositol system, binding assays with [3H]5-HT, used to identify 5-HT1 sites, and 5-HT as competitor have shown minimal binding present. In contrast, assays using [3H]LSD, which has affinity for both 5-HT1 and 5-HT2 sites, and mianserin as competitor showed binding sites to be present to a much greater extent. When 5-HT was used as competitor for [3H]LSD, once again minimal binding was present. Based on the results of the [3H]5-HT and [3H]LSD binding assays, it was anticipated that binding of [3H]ketanserin, a specific 5-HT2 ligand, would be robust. However, a low level of [3H]ketanserin sites was found. This initial pharmacological profile may indicate the presence in *A. suum* muscle of several 5-HT receptor subtypes similar to mammalian 5-HT receptor subtypes and/or a novel non-5-HT1 receptor which functions through the cAMP system. Further comparisons of the *A. suum* 5-HT receptor to the mammalian 5-HT receptor subtypes are being carried out.

427.19

MDL 100,907, (+)-α-(2,3-dimethoxyphenyl)-1-[2-(4-fluorophenylethyl)]-4-piperidinemethanol, a potent, chiral, 5-HT$_2$ receptor antagonist. M. Dudley, A. Ogden*, A. Carr*, T. Nieduzak* and J. Kehne. Merrell Dow Research Institute, Cincinnati, OH 45215

The racemic compound MDL 11,939 (α-phenyl-1-(2-phenylethyl)-4-piperidinemethanol) has been reported previously as a potent and selective 5-HT$_2$ antagonist (Dudley et al. Drug Dev. Research 13:29, 1988). Synthesis of the enantiomers, MDL 28,233, the (-)-isomer, and MDL 28,727, the (+)-isomer showed that the potent 5-HT$_2$ receptor antagonism was associated with the (+)-isomer. MDL 28,727 had an IC_{50} value at rat cortical 5-HT$_2$ receptor of 4.2 nM compared to 190 nM and 10 nM for MDL 28,233 and MDL 11,939, respectively. Further investigation of possible substituents on both aryl nuclei to increase potency resulted in the racemic MDL 100,151 ((±)-α-(2,3-dimethoxyphenyl)-1-[2-(4-fluorophenylethyl)]-4-piperidinemethanol), which was resolved into MDL 100,907, the (+)-isomer and MDL 100,009, the (-)-isomer. In initial binding studies with [^{3}H]spiroperidol in rat cortical membranes, MDL 100,151, MDL 100,907 and MDL 100,009 had IC_{50} values of 1 nM, 0.4 nM and 12 nM respectively. MDL 100,907 blocked the 5-methoxy-N,N-dimethyltryptamine-induced head twitch in mice with an ED_{50} of 0.03 mg/kg, i.p. MDL 100,907 was 130-fold selective for the 5-HT$_2$ receptor over the 5-HT$_{1c}$ receptor. In conclusion, MDL 100,907 is a stereospecific, potent and selective 5-HT$_2$ receptor antagonist.

427.20

DIFFERENTIATION OF 8-OH-DPAT AND IPSAPIRONE IN RAT MODELS OF 5-HT$_{1A}$ RECEPTOR FUNCTION. J. De Vry*, R. Schreiber*, J.M. Greuel, E. Horvàth*, T. Glaser. Institute for Neurobiology, Troponwerke GmbH & Co. KG, Berliner Str. 156, 5000 Köln 80, F.R.G.

8-OH-DPAT (D) and ipsapirone (I) bind 5-HT$_{1A}$ receptors located presynaptically (PREsyn) in the raphe nuclei and postsynaptically (POSTsyn), predominantly in the limbic system. In the forskolin stimulated adenylate cyclase model, the 5-HT behavioral syndrome test, and the circling test (after unilateral application of 5,7-DHT in the substantia nigra), three models of POSTsyn 5-HT$_{1A}$ receptor activation, D and I are characterised as full and partial agonists, respectively. D and I completely suppress raphe 5-HT cell firing in slice preparations and in vivo; suggesting that both compounds are PREsyn 5-HT$_{1A}$ agonists. In drug discrimination (D cue) and hypothermia, models which presumably involve PRE -and POSTsyn 5-HT$_{1A}$ receptors, D and I are full agonists. To conclude, it appears that D is a full agonist at PRE- and POSTsyn 5-HT$_{1A}$ receptors; whereas I is a full and partial agonist at PRE- and POSTsyn receptors, respectively. However, the finding that (1) I, like D, completely generalizes to the D cue when applied bilaterally in the dorsal hippocampus, and (2) both I and D retain full agonistic activity in the hyperthermia model after 5,7-DHT induced lesion of the brain 5-HT system, suggests that there are brain regions where both compounds are full agonists at POSTsyn 5-HT$_{1A}$ receptors.

RECEPTOR MODULATION: UP AND DOWN REGULATION I

428.1

CHRONIC ETHANOL AFFECTS PERIPHERAL-TYPE BENZODIAZEPINE RECEPTORS: REGIONAL DIFFERENCES AND ROLE OF PHYSICAL DEPENDENCE. P.J. Syapin, P.A. Assanah*, L.S. Kobayashi*, B. L. Jones*, D.A. Finn, and R.L. Alkana. U.S.C. Health Sciences Ctr., Los Angeles, California 90033

Mice and rats made physically dependent on ethanol have increased numbers of brain peripheral-type benzodiazepine receptors (PBR). To further characterize this change PBR were measured in C57BL/6J mice which consumed 3.5% ethanol (nondependent) or 7% ethanol (physically dependent). Binding of [^{3}H]Ro5-4864 to olfactory bulb, pons-medulla, cerebellum, and anterior and posterior cerebrum membranes indicated increased PBR in posterior cerebrum of physically dependent mice and no changes in other regions. Nondependent mice had reduced PBR in olfactory bulbs with no change in other regions. Cardiac and spleen PBR were not altered, although organ weights decreased. Thymus PBR increased in nondependent mice and tended to increase in dependent mice, despite pronounced thymic involution. K_D values were not significantly altered by chronic ethanol exposure. These results suggest that physical dependence upregulates PBR in posterior cerebrum brain regions and "normalizes" changes in olfactory bulb PBR found in nondependent mice. Whether alterations in brain PBR function accompany chronic ethanol exposure requires further study. (NIAAA grant AA07351)

428.2

SHORT AND LONG TERM TOLERANCE TO ABECARNIL, A BETA-CARBOLINE BENZODIAZEPINE RECEPTOR LIGAND: ANTAGONISM OF PTZ CONVULSION THRESHOLD VS RECEPTOR OCCUPATION AND PLASMA LEVEL IN THE MOUSE. H.H. Schneider, L. Turski, W. Krause* and D.N. Stephens*. Dept. of Neuropsychopharmacology, Schering AG, 1000 Berlin 65, West Germany.

Abecarnil (ABC) is a non-sedative anxiolytic β-carboline (Stephens, D.N. et al., *JPET* 253: 1990). For the investigation of tolerance phenomena we used the anticonvulsant properties of ABC (Turski, L. et al., *JPET* 253: 1990). Repeated treatment with ABC (15 mg/kg ip, twice daily for 12 days) led to a slight reduction in its potency to antagonize the induction of clonic seizures by pentylenetetrazol (PTZ) given iv. The seizure threshold at 5 mg ABC/kg ip, 30 min declined from 152 mg PTZ/kg (acute) to 122 mg PTZ/kg (subchronic). For short term tolerance, the PTZ seizure threshold was determined following ABC, 2 mg/kg ip between 10 and 320 min. To determine the degree of benzodiazepine receptor occupation by ABC the animal received ^{3}H-lormetazepam, 7.4 MBq/kg sc, 20 min before decapitation. Immediately following the PTZ threshold determination the animal was decapitated. Blood was collected for HPLC estimation of ABC, and the forebrain homogenized in cold buffer, and aliquots filtered for determination of bound radioactivity. The time course of the three parameters went roughly parallel, with peak values at 40 min. However, when the correlation of PTZ antagonism and benzodiazepine receptor occupation were plotted for all time points it became evident that at the same degree of receptor occupation, ABC was clearly more effective in the early phase (10-40 min) than in the second, elimination phase. This acute potency shift was compared to that following Alprazolam. This "hysteresis" effect may be useful for the prediction of tolerance development.

428.3

PSYCHOLOGICAL AND GENDER INFLUENCES ON THE STRESS-INDUCED DECREASE IN RENAL PERIPHERAL BENZODIAZEPINE RECEPTORS IN RAT. R.C.Drugan, A.P.Stringer, & P.V.Holmes. Schrier Research Laboratory, Dept. of Psychology, Brown University, Providence, RI 02912.

Earlier research has shown that exposure to environmental stress results in changes in the density of peripheral benzodiazepine receptors (PBR) in both central nervous system (CNS) and peripheral tissues. We have previously evaluated the characteristics of physical stress necessary for these changes, however, neither the psychological dimensions of stress nor gender influences have been investigated.

We now report that controllability and predictability of tailshock stress do not result in a modification of the stress-induced changes in renal PBR. Further, psychological stress alone, using a conditioned fear paradigm, does not produce a change in the binding of [3H]RO5-4864 to PBR in comparison to controls. However, administration of an anesthetic dose of sodium pentobarbital (60mg/kg, i.p.) prior to the stress completely blocks the decrease in PBR indicating the importance of consciousness during stress. Finally, we also report gender differences in the response of renal PBR to inescapable stress. Female rats show an attenuated decrease in PBR (23%) as compared to male rats (50%). These data suggest both neural and hormonal mediation of the stress-induced alterations in renal PBR. SUPPORTED BY: NIMH grant #MH44034-01A1 & an Alfred P. Sloan Research Fellowship to Robert C. Drugan.

428.4

REGIONAL CHANGES IN RAT BRAIN BENZODIAZEPINE RECEPTORS FOLLOWING SEIZURES. J.L. Daval, M.C. Werck* and P. Vert*, INSERM U272, 24-30 rue Lionnois, 54013 NANCY, FRANCE.

Benzodiazepines (BZ) are psychoactive substances classically used for their anticonvulsant properties as well in neonates as in adults. In a previous work, we have shown that seizures lead to an age-dependent upregulation of central BZ binding sites measured in isolated cerebral membranes (Life Sci., 41: 1685, 1987). However, no information was available concerning the regional changes in the receptor density. Therefore, the effects of bicuculline-induced seizures were investigated by quantitative autoradiography of central BZ receptors in developing rats and in adults. Animals received an i.p. injection of either saline or bicuculline and were sacrificed 30 min later. Brain sections were incubated with |3H|flunitrazepam. Seizures induced a significant increase in BZ receptors in 39 out of the 46 brain regions studied, with a marked enhancement in structures that mediate seizure activity such as substantia nigra (+43% and +21% in 5-day-old rat and in adult respectively), amygdala (+20% and +12%), septum (+26% and +15%), hippocampus (+42% and +34%), as well as in cortices. Moreover, the addition of 10^{-4} M GABA into the incubation medium increased BZ binding in a similar manner whether rats were given bicuculline or saline, suggesting that increased BZ sites are also functionally linked to GABA receptors. The age-dependent postictal increase in BZ receptors might be an adaptative phenomenon for protection against recurrent seizures.

428.5

HOMOLOGOUS AND HETEROLOGOUS UNCOUPLING OF THE $GABA_A$ RECEPTOR COMPLEX BY GABA, 3α-OH-DHP, FLURAZEPAM AND PENTOBARBITAL IN CULTURE. L.K. Friedman, T.T. Gibbs, and D.H. Farb, Dept. Anatomy & Cell Biology, SUNY Health Science Center at Brooklyn, N.Y. 11203.

We have previously shown that chronic treatment with GABA, certain steroids, benzodiazepines, or barbiturates produces a reduction in the allosteric coupling between the benzodiazepine recognition site and other sites on the $GABA_A$ receptor which is refered to as "uncoupling". Here we describe homologous and heterologous uncoupling induced by chronic (48 h) exposure of neuronal cultures to GABA (1mM), 5β-pregnan-3α-ol-20-one (3α-OH-DHP) (10μM), flurazepam (50μM), or pentobarbital (PB) (200μM). Allosteric interactions were measured by the effect of 5μM 3α-OH-DHP, 300 μM pentobarbital, or 10 μM GABA on reversible binding of 1 nM ^{3}H-flunitrazepam. While chronic steroid treatment blocked potentiation of ^{3}H-flunitrazepam binding by pentobarbital and 3α-OH-DHP, potentiation by GABA was only partially reduced (by 40%). Chronic GABA treatment greatly reduced (by 80%) PB-stimulation of ^{3}H-flunitrazepam binding and modestly reduced (by 30%) GABA enhancement of ^{3}H-flunitrazepam binding, while steroid-stimulated ^{3}H-flunitrazepam binding was unaffected. In contrast, chronic flurazepam treatment reduced the potentiation produced by all 3 compounds by a similar degree. Finally, pentobarbital exposure decreased both PB and GABA stimulation of ^{3}H-flunitrazepam binding, but had little effect on 3α-OH-DHP stimulation of ^{3}H-flunitrazepam binding. Coincubation of cultures with 3α-OH-DHP and SR-95531, a selective $GABA_A$ antagonist, reduced the steroid-induced decrease of GABA potentiation but did not prevent the loss of 3α-OH-DHP stimulation of ^{3}H-flunitrazepam binding. These results suggest the existence of multiple modes of uncoupling which are most likely mediated through different sites.

428.6

EFFECT OF DESIPRAMINE (DMI) ON HIGH FREQUENCY NEUROTRANSMISSION (HFN) AND RECURRENT INHIBITION (RI) IN RAT HIPPOCAMPAL SLICE. F.A. Dijcks*, J.H. Couvée* and G.S.F. Ruigt, Organon int, CNS Pharmacology Dept., POB 20, 5340 BH Oss, The Netherlands.

Tricyclic antidepressants are known to have specific acute interactions with several neurotransmitter systems in rat brain. However, adaptive changes to chronic antidepressant treatment, rather than the acute pharmacological properties of the drugs, seem to contribute to the understanding of their mechanism(s) of action. In the present experiment we tested the effects of DMI by acute treatment in vitro (10 μM), and single dose and long-term treatment in vivo (10 mg/kg) on the excitatory and inhibitory neurotransmission in rat hippocampal slice. We recorded ortho- and antidromically evoked field potentials in stratum radiatum (fEPSP) and pyramidale (PS) of area CA_1. HFN was tested by evoking field potentials at 20 Hz. RI was tested by antidromic activation of the pyramidal cells prior to an orthodromic test pulse. We demonstrate that a frequency-dependent inhibiton by 10 μM DMI in vitro is observed for the antidromic PS and for the fEPSP although at high stimulus intensity the fEPSP is augmented. Pretreatment of the rats with DMI does not affect the frequency-dependent inhibition of the fEPSP and PS by DMI in vitro. Acute DMI did not affect RI (as % decrease of the orthodromic test pulse) i.e. RI dropped from 37 + 8 to 35 + 6% inhibition. Single dose or long-term pretreatment with DMI caused an increase in RI resp. to 53 + 4% (two-tailed t-test: $P<.051$) and 62 + 5% ($P<.014$). We show that HFN is inhibited only through the acute action of DMI although the magnitude of the effect seems to depend on stimulus intensity and that RI is augmented by in vivo and not in vitro DMI treatment.

428.7

EFFECTS OF CHRONIC ANTIDEPRESSANT DRUG ADMINISTRATION ON GABA B RECEPTORS. D.J. McManus*, A.J.Greenshaw*, G.B. Baker AND T.B. Wishart. Neurochemical Research Unit, Dept. of Psychiatry, Univ. of Alberta, Edmonton, Alberta, Canada, T6G 2B7 and Dept. of Psychology, Univ. of Saskatchewan, Saskatoon, Saskatchewan, Canada, S7N 0W0.

Effects of chronic administration of antidepressant drugs on $GABA_B$ receptors have been assessed both in vivo and ex vivo. Tricyclics [imipramine (IMI) 30 mg/kg/d and desipramine (DMI) 10 mg/kg/d] or monoamine oxidase (MAO) inhibitors [(+/-)-tranylcypromine (TCP) 1 mg/kg/d and phenelzine (PLZ) 10 mg/kg/d] (all doses expressed as salts) were administered to male Sprague-Dawley rats (N=10 per group), by constant infusion via Alzet 2ML4 osmotic minipumps for 28 d. Pumps were implanted sc in the interscapular region. On days 21 and 22 the motor-supressant actions of the $GABA_B$ receptor agonists (+/-)-baclofen (5 mg/kg ip) and progabide (50 mg/kg ip) were assessed as an in vivo measure of $GABA_B$ receptor sensitivity. On day 28 the animals were killed and their brains used for measurement of drug levels and MAO activity. Tissue from the frontal cortex was used to assess the number (Bmax) and affinity (Kd) of $GABA_B$ receptors using [^{3}H]-GABA as the radiolabelled ligand. Non-specific binding was defined using (+/-)-baclofen. All of the drugs tested failed to induce an increase in the in vivo responsiveness of animals to (+/-)-baclofen or progabide. [3H]-GABA binding was unchanged for rats receiving PLZ, DMI or IMI ($p<0.05$, vs VEH controls). Only TCP-treated animals showed an increased Bmax relative to controls. No effect on Kd was observed in any drug group. These data do not support the proposal that an increase in the number of GABA B receptors is a common effect of chronic antidepressant drug treatment.

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428.8

LONG-TERM TREATMENT WITH LITHIUM AND CARBAMAZEPINE ATTENUATES CARBACHOL-INDUCED PHOSPHOINOSITIDE TURNOVER IN NEURONS. X.-M. Gao, F. Fukamauchi, C. Hough and D.-M. Chuang. Biological Psychiatry Branch, NIMH, Bethesda, MD

We have studied long-term effects of lithium and carbamazepine (CBZ) on muscarinic receptor (mAChR)-mediated phosphoinositide (PI) turnover in cultured cerebellar granule cells. Three days' exposure of cells to lithium induced a biphasic change in carbachol-induced accumulation of ^{3}H-inositol monophosphate (IP_1). The accumulation was increased up to 2 mM of lithium but inhibited by higher concentrations of this ion. Exposure of cells to 5 mM lithium for more than 3 days resulted in marked inhibition of the PI response to carbachol with about 75% inhibition at day 7. Three days' exposure to CBZ also induced a decrease of carbachol-induced ^{3}H-IP_1 accumulation with an IC_{50} of around 30 μM and virtually no effect on basal activity. This CBZ effect was due to a decrease in the maximal response to carbachol. The decrease was about 30% at day 3 and largely reversed at day 6. Cell-surface mAChR receptors assessed by binding with ^{3}H-NMS were decreased by long-term exposure to lithium (3 mM) but not by CBZ (30 μM). Moreover, Northern blot hybridization revealed that long-term lithium treatment decreased m3-mAChR mRNA level with no significant change on levels of total RNA, m2-AChR mRNA and β-actin mRNA. Thus, both lithium and CBZ have long-term converging effects on m3-AChR function in granule cells; however, distinct mechanisms appear to be involved in this receptor modulation.

428.9

ROLE OF CYTOSKELETONS IN THE REGULATION OF mRNA OF m3-MUSCARINIC ACETYLCHOLINE RECEPTORS. F. Fukamauchi, C. Hough and D.-M. Chuang. Biological Psychiatry Branch, NIMH, Bethesda, MD 20892.

The regulation of carbachol (CCh)-induced muscarinic acetylcholine receptor (mAChR) mRNA by cytoskeletal agents was studied in cultured cerebellar granule cells using Northern blot hybridization. In 8-day-old cultures, m2-mAChR mRNA levels were about 50 and 70% of the control at 2 and 4 hours after treatment with 100 μM CCh, respectively. Levels of m3-mAChR mRNA were about 75 and 50% of the control at 4 and 8 hours, respectively. Exposure of cells to methylamine alone (10 mM), an inhibitor of endocytosis, for 8 hours led to down-regulation of m3-mAChR mRNA, but did not affect CCh-induced m3-mAChR mRNA down-regulation. Bacitracin (2 mg/ml), an inhibitor of transglutaminase, did not change levels of basal and CCh-down-regulated m3-mAChR mRNA. However, exposure to colchicine alone (1-100 μM), a microtubule disrupting agent, for 4 hours led to down-regulation of m2- and m3-mAChR mRNA in a dose-dependent manner. Total RNA was decreased and m3-mAChR mRNA was not detected at 24 hours after treatment with 10 μM colchicine. Furthermore, treatment of cells for 8 hours with 10 μM colchicine enhanced the down-regulation of m3-mAChR mRNA induced by 100 μM of CCh. An antimicrofilament agent, cytochalasin B at 5 μM/ml, had no effect on the CCh-induced mAChR mRNA down-regulation. These results suggest that mAChR mRNA down-regulation may involve the function of microtubular elements.

428.10

PROTEIN KINASE C INHIBITS THE DEPOLARIZATION MEDIATED BY α_1-ADRENERGIC RECEPTORS IN RAT DORSAL RAPHE NEURONS. T. J. Grudt* and J. T. Williams. Vollum Institute, Oregon Health Sciences University, Portland, OR 97201.

Intracellular recordings were made from dorsal raphe (DR) neurons in slices made from rat brain. The α_1-adrenergic receptor agonist phenylephrine (PE) depolarized DR neurons. A protein kinase C activator, phorbol 12,13-dibutyrate (PDBU), had no effect on membrane potential by itself but inhibited the PE induced depolarization in a dose-dependent manner (EC50 = 28 nM). The inactive phorbol ester phorbol 12,13-didecanoate had no effect. The PDBU induced inhibition was reversed by the non-specific protein kinase inhibitor staurosporin (3 μM). Agonist-induced desensitization of the α_1-adrenergic increase in phospholipid turnover has been reported and is mimicked by the application of phorbol esters (Leeb-Lundberg et al. J.Bio.Chem. 262:3098, 1987). Our results are consistent with α_1-adrenergic receptor desensitization mediated by protein kinase C. Since α_1-adrenergic receptor activation leads to formation of diacylglycerol and activation of protein kinase C, this may be an example of feedback inhibition.

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428.11

COMPARISON OF AGONIST AND ANTAGONIST COMPETITION FOR [^{3}H]RAUWOLSCINE (RAU) BINDING TO α_2-ADRENERGIC RECEPTORS (A2AR) OF INTACT CHINESE HAMSTER OVARY (CHO) CELLS AND ISOLATED MEMBRANES. P.E. Shreve, M.L. Toews, and D.B. Bylund. Dept. of Pharmacology, Univ. of Nebraska Med. Ctr., Omaha, NE 68198.

β-adrenergic (BAR) agonists exhibit markedly lower binding affinities for BAR in intact cells as compared to isolated membranes. In contrast, BAR antagonists have similar affinities for BAR in intact cells and membranes. This phenomenon has been similarly observed for α_1-adrenergic and muscarinic receptors (J. Pharmacol. Exp. Ther. 251(1): 63-70, 1989). These changes in binding properties of agonists may be related to the process of desensitization. The purpose of this study was to compare the binding affinities of agonists and antagonists to A2AR in intact cells and isolated membranes of CHO cells which have been stably transfected with the cDNA for A2AR. Pharmacological studies indicated that [^{3}H]RAU, an A2AR antagonist, exhibited high affinity for both intact CHO cells and CHO membranes with K_Ds of 1.6 and 0.53 nM, respectively. Norepinephrine (NE) and prazosin (PRAZ) have similar binding affinities for A2AR in isolated CHO membranes and were chosen as the agonist and antagonist respectively in the present study. The Ki values (n=3-4) were determined by computer-assisted nonlinear curve fitting. NE and PRAZ had Ki values of 0.8 and 0.4 μM respectively in isolated CHO membranes. The respective Ki values of NE and PRAZ were 74 and 1.3 μM in intact CHO cells. Thus NE had a >90 fold lower affinity in intact CHO cells as compared to CHO membranes. In contrast, PRAZ had only a 3 fold lower affinity in intact CHO cells as compared to CHO membranes. Therefore, the agonist exhibited an apparent binding affinity for A2AR in intact CHO cells which was markedly lower than its affinity in CHO membranes. This difference in agonist binding affinity between intact cells and isolated membranes is similar to that observed for other receptor systems and may play a role in A2AR agonist induced desensitization. This research is supported by NIH grant GM37664.

428.12

DIFFERENCES IN RESPONSES TO CHRONIC RESERPINE OF BETA-ADRENERGIC RECEPTORS IN NEOCORTEX AND LIMBIC CORTEX. L.J. Grimm, K.J. Kellar[1]and D.C. Perry. Depts. of Pharmacology, George Washington and [1]Georgetown Univ. Medical School, Washington, D.C. 20037.

We have previously used autoradiography to demonstrate that chronic reserpine administration increases β-adrenergic receptor binding in many brain regions (FASEB J. 4: A459, 1990). In this study we examined for reserpine-induced changes in β-stimulated cAMP production, comparing two general cortical regions. Rats were treated for 15 days with reserpine i.p.: 0.5 mg/kg days 1 & 2, and 0.25 mg/kg thereafter. Rats were sacrificed 24 hr after the last dose and brains bisected sagitally; half was frozen for binding analysis, and half used for cAMP analysis. The cortex was dissected free and divided by a knife cut parallel to the rhinal sulcus into an "upper" region (essentially neocortex) and a "lower" region (essentially limbic cortex, including rhinal cortices and amygdala). 350 μ slices were prepared and stimulated with various agonists; cAMP levels were measured by RIA. Stimulation (3-8 fold) was seen with norepinephrine [NE], isoproterenol [ISO], ISO + 6-fluoronorepinephrine [6FNE], and forskolin, but not 6FNE alone. The stimulation by NE, ISO, and 6FNE + ISO, but not forskolin, was increased after chronic reserpine. The enhancement by reserpine treatment of ISO and NE stimulation (but not ISO + 6FNE stimulation) was significantly higher in the "upper" region (70-110%) compared to the "lower" regions (20-50%). Saturation binding of [^{3}H]CGP-12177 done on the remaining half brain, similarly dissected, showed increases in B_{max} of 28-33% after chronic reserpine, limited to β-1 receptors. In contrast to cAMP stimulation, no differences between upper and lower regions were seen. Thus the effect of chronic reserpine on β-stimulated cAMP, but not β-binding, is greater in neocortex than limbic cortex. These regional differences suggest a difference in the efficiency of coupling of β-1 receptors to adenylate cyclase in neocortex compared to limbic cortex.

428.13

EFFECTS OF REPEATED ELECTROCONVULSIVE SHOCK (ECS) ON SEROTONIN-1A (5-HT-1A) RECEPTOR BINDING AND RECEPTOR-MEDIATED HYPOTHERMIA IN RAT. C.A. Stockmeier and G.A. Gudelsky, Departments of Psychiatry and Neuroscience, Case Western Reserve University, Cleveland, OH 44106.

5-HT-1A receptors may be involved in the mechanism of action of antidepressant treatments. Chronic antidepressant drugs or ECS attenuate 8-hydroxy-2-(di-n-propylaminotetralin) (8-OH-DPAT)-induced hypothermia (Goodwin et al., 1987). Male rats were handled or received ECS for either one or ten days, and were killed two days later. Ten days of ECS decreased beta-adrenergic receptor binding in frontal cortex. However, [^{3}H]8-OH-DPAT binding in cortex or hippocampus was not affected by repeated ECS. In hypothalamus, ten days (but not one day) of ECS significantly decreased [^{3}H]8-OH-DPAT binding. In addition, 48 hr following 10 days of ECS, the hypothermic response to 8-OH-DPAT (0.1 mg/kg, sc) was attenuated in ECS-treated rats compared with handled controls. The hypothermic response to 8-OH-DPAT was returned to normal at 14 days after the last of 10 treatments with ECS. A single treatment with ECS did not affect the hypothermic response to 8-OH-DPAT. The ECS-induced decrease in [^{3}H]8-OH-DPAT binding in the hypothalamus may be responsible for the ECS-induced attenuation of the hypothermic response to 8-OH-DPAT. Supported by NS24523 and MH41684.

428.14

REGULATION OF THE 5-HT$_2$ RECEPTOR IN VIVO: AN AUTORADIOGRAPHIC STUDY. **M. Hauptmann* and A. Frazer, Dept. of Vet. Affairs Med. Ctr. & Univ. of Pa. Sch. of Med., Phila., PA 19104.**

The 5-HT$_2$ receptor has regulatory properties different from those exhibited by many other neurotransmitter receptors. Previous studies of adaptive changes in 5-HT$_2$ receptors after chronic administration of antidepressants have focused on radioligand binding in homogenates of rat frontal cortex. We have used the technique of quantitative autoradiography to measure the effect of either mianserin, monoamine oxidase inhibitors (MAOI's) or a selective serotonin uptake inhibitor, citalopram, on the binding of ^{3}H-ketanserin in different regions of rat brain. Acute administration of mianserin (10mg/kg) decreased ^{3}H-ketanserin binding to 5-HT$_2$ receptors in all layers of the frontal cortex and in hypothalamic and amygdaloid nuclei. Even though chronic treatment with mianserin (15mg/kg,twice daily for 21 days) caused a greater decrease in ^{3}H-ketanserin binding in the frontal cortex than acute treatment did, it had no effect on ^{3}H-ketanserin binding in the dorsomedial and ventromedial hypothalamic nuclei. The MAOI's, tranylcypromine (5mg/kg, once daily for 21 days) and clorgyline (1mg/kg once daily for 21 days) caused a significant (about 30%) decrease in 5-HT$_2$ receptor density in layer IV of the frontal cortex, but not in any subcortical areas. Citalopram (20mg/kg, once daily for 21 days) had no effect on ^{3}H-ketanserin binding in any areas of the brain studied. It seems that ^{3}H-ketanserin binding in the frontal cortex is somewhat more amenable to regulatory influences than such binding measured elsewhere in brain. Care should be exercised in extrapolating data obtained on the regulation of 5-HT$_2$ receptors in cortex to other regions of brain. **(Supported by Research Funds from the Dept. of Vet. Affairs & USPHS grant MH 29094).**

428.15

EFFECTS OF REPEATED ADMINISTRATION OF ANTIDEPRESSANTS ON SEROTONIN (5-HT) UPTAKE SITES MEASURED USING [^{3}H]CYANOIMIPRAMINE (^{3}H-CN-IMI) AUTORADIOGRAPHY. G.B. Kovachich, D.J. Brunswick, C.E. Aronson and A. Frazer. Univ. of PA Sch. of Med. & Dept. Vet. Affairs Med. Ctr., Phila., PA 19104.

To determine if repeated administration of antidepressants to rats regulate 5-HT uptake sites, the binding of [^{3}H]CN-IMI was measured using quantitative autoradiography. Under our assay conditions, this radioligand binds specifically, and with high affinity, to 5-HT uptake sites (*Brain Res.*, 454:78, 1988). All antidepressant drugs were administered for 21 days except citalopram (14 days) and were given i.p. except for deprenyl (s.c.). The drugs used were: the 5-HT uptake inhibitors sertraline (5mg/kg, daily) and citalopam (20mg/kg, daily); the norepinephrine uptake inhibitor protriptyline (15mg/kg, twice daily); the α_2-adrenergic antagonist mianserin (10mg/ kg, twice daily); the non-selective MAO inhibitor phenelzine (5mg/kg, twice daily) and the type B MAO inhibitor deprenyl (0.25 mg/kg, daily). Rats were killed 18 hrs after the last injection of drug. All structures examined were from brain slices taken at the level of plate 30 of the atlas of Paxinos and Watson (1986). Administration of citalopram or protriptyline had no effect on [^{3}H]CN-IMI binding in any brain region examined. Treatment with sertraline, phenelzine, deprenyl or mianserin caused some regionally specific alterations in the number of binding sites, but the effects were modest in size (10-20%) and no common effects were seen. Thus, no consistent regulatory effects associated with 5-HT uptake are produced by antidepressant drugs. **(Supported by research funds from the Department of Veterans Affairs.)**

428.16

LONG-TERM BUT NOT SHORT-TERM LITHIUM TREATMENT POTENTIATES m-CPP INDUCED CHANGES IN PLASMA PROLACTIN AND CORTICOSTERONE BUT NOT GROWTH HORMONE LEVELS IN RATS. C.S. Aulakh*, J.L. Hill*, N.A. Garrick and D.L. Murphy*. Lab. of Clinical Science, NIMH, Bethesda, MD 20892

The lithium ion is effective clinically for the treatment of acute manic illness, prophylaxis in manic-depressive (bipolar) and unipolar depressive disorders, and in conjunction with antidepressant drugs for the treatment of resistant depressive illness (DeMontigny et al 1983; Prien et al 1984; Price et al 1990). The therapeutic effects of antidepressant and antimanic drugs are manifested after only two or more weeks of administration. By using m-chlorophenylpiperazine (m-CPP, a postsynaptic 5-HT1 receptor agonist) as a challenge agent, we investigated functional adaptational changes in the serotonergic neurotransmitter mechanisms regulating prolactin, corticosterone and growth hormone secretion following long-term lithium treatment in rats. Male Wistar rats were given rat chow containing lithium carbonate (1.65g/kg) for 28 days. Saline or various doses of m-CPP were injected intravenously (11-11:15 a.m.) at least 48 hours after the cannulas were implanted in the blood vessel; blood samples were drawn 30 minutes after saline or m-CPP injection. Long-term (21-23 days) lithium treatment potentiated m-CPP-induced increases in plasma prolactin and corticosterone levels but did not have any effect on m-CPP-induced decreases in plasma growth hormone levels. Short-term (2-4 days) lithium treatment attenuated m-CPP's effect on plasma corticosterone levels without any effect on plasma prolactin or growth hormone levels. These findings document an enhancement of some aspects of brain 5-HT function following long-term lithium treatment and support other data suggesting that lithium's serotonergic actions may be relevant to its therapeutic efficacy.

GABA$_B$ RECEPTORS

429.1

MODULATION OF INHIBITORY SYNAPTIC TRANSMISSION IN HIPPOCAMPAL SLICES BY ACTIVATION OF PRESYNAPTIC GABA-B RECEPTORS ? I.M.Stanford*, J.E.Chad and H.V.Wheal. Department of Neurophysiology, University of Southampton, Bassett Crescent East, Southampton SO9 3TU, UK. (Sponsored by the Brain Research Association, UK).

CA1 pyramidal cell responses to paired stimuli were recorded extracellularly from the rat hippocampal slice preparation. The inhibition produced by the initial (conditioning) stimuli was quantified by measuring the response to the second (test) stimuli, and comparing it to the response to the initial stimuli.

Experiments were performed to measure the effect of interpulse interval on the inhibition observed. Orthodromically evoked response pairs for control slices (n=18) show inhibition at short interpulse intervals (0-30ms) and also at longer intervals (200-400ms), whereas antidromically evoked response pairs show no inhibition at these intervals. As has been previously reported, antagonists of GABA A receptors (bicuculline 1uM) enhanced both responses, with a reduction in paired pulse inhibition (measured as the ratio of test to conditioning responses) at short intervals but increased inhibition at longer interpulse intervals (n=7).

Antagonists of the GABA B receptor (phaclofen 1mM) had little effect on the conditioning response but prevented the reduction of the test response at long interpulse intervals, thus reducing paired pulse inhibition (n=7). Whereas the GABA B agonist baclofen (1uM) reduced both responses whilst attenuating paired pulse inhibition (n=7). Some proportion of the decrease in paired pulse inhibition can be attributed to the reduction in conditioning response (to 35% of control values). However, extrapolation from control experiments on the effects of changes in conditioning response amplitude, produced by varying stimulus intensities, would lead to the prediction of a smaller decrease in late inhibition. This leaves open the possibility that direct action of baclofen on presynaptic GABA B receptors, located on inhibitory interneurones, may contribute to the reduction in paired pulse inhibition.

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429.2

2-Hydroxy-Saclofen Reduces the Depression of IPSCs Induced by a Variety of Stimulation Patterns in Rat Hippocampus. C.H. Davies* and G.L. Collingridge* (SPON: Brain Research Association) Department of Pharmacology, University of Bristol, Bristol, BS8 1TD, U.K.

Recently we reported that at an interstimulus interval of 100 ms paired-pulse depression of monosynaptic IPSCs was abolished by the GABAB receptor antagonist 2-hydroxy-saclofen (Davies et al, 1990, J. Physiol. 424 513-531). We have now examined the effect of 2-hydroxy-saclofen on a wider range of intervals (25-2500 ms) as well as its effect on more complex stimulation paradigms. In the presence of D-AP5 (40-80 uM) and CNQX (20 uM) stimulation in stratum radiatum evoked monosynaptic IPSCs. 2-hydroxy-saclofen (400 uM) abolished paired-pulse depression of IPSCs at all interstimulus intervals tested. Complete block of the depression induced by stimulus trains (5-10 Hz for 1 s) was also achieved although at frequencies of >10 Hz 2-hydroxy-saclofen only partially reversed the depression of IPSCs. We have also tested 2-hydroxy-saclofen on IPSCs evoked by primed-pulse (e.g., 2 shocks of 20-100 Hz preceded 140-170 ms by a single shock) and patterned-burst (e.g., 5 bursts of 4 shocks of 100 Hz delivered at 200 ms intervals) stimulation paradigms. In all cases 2-hydroxy-saclofen partially reversed the depression of synaptic inhibition.

429.3

BICUCULLINE-RESISTANT GABA MEDIATED EVENTS DISCLOSED BY THE GABA$_B$ ANTAGONIST 2-HYDROXY-SACLOFEN. Jose M. Solis and Roger A. Nicoll Dept. Pharmacol., Univ.California, San Francisco, CA 94143

In the hippocampus the GABA$_B$ antagonist phaclofen is a good inhibitor of both the postsynaptic baclofen response and the slow IPSP, but only inhibits partially the bicuculline-resistant response evoked by GABA. It has recently been reported that 2-hydroxy-saclofen (2-OH-S), a more potent GABA$_B$ antagonist, is able to block the slow IPSP in the hippocampus (Lambert et al., 107, 125-128, 1989). We have used single electrode voltage-clamp in CA1 pyramidal cells of rat hippocampal slices. While high concentrations (500 μM) of 2-OH-S completely inhibited the outward current evoked by (±) baclofen iontophoresis, 2-OH-S only reduced the bicuculline-resistant GABA currents by 30.4±8.6% and did not have any effect on the serotonin-evoked outward current. In addition 2-OH-S (500 μM) also blocked the "pure" slow IPSP evoked by local stimulation in the stratum radiatum in the presence of glutamate receptor antagonists. The slow IPSP appears to be mediated by synaptically released GABA since its time course was prolonged in the presence of the GABA uptake inhibitors cis-4-hydroxynipecotic and DL-2,4 diamino-N-butyric acids.

In summary, these results support the proposal that the slow IPSP is generated by GABA acting on GABA$_B$ receptors. However, the fact that part of the outward current evoked by exogenous GABA is resistant to GABA$_A$ and GABA$_B$ antagonists raises the possibility that a third type of GABA receptor exists on hippocampal pyramidal cells.

429.4

2-HYDROXYSACLOFEN: AN ANTAGONIST AT PRE- AND POSTSYNAPTIC GABA$_B$ RECEPTORS IN THE RAT HIPPOCAMPUS. N.L. Harrison, D.M. Lovinger[1], N.A. Lambert[2], T.J. Teyler[2] and D.I.B. Kerr[3] Dept. of Anesthesia and Critical Care, The Univ. of Chicago, Chicago, IL, [1]Lab. of Phys. and Pharm. Studies, NIAAA, Rockville, MD, [2]Dept. of Neurobiology, NEOUCOM, Rootstown, OH, and [3]Dept. of Anesthesia and Intensive Care, The Univ. of Adelaide, Australia.

We studied the actions of the GABA$_B$ receptor antagonist 2-hydroxysaclofen (2-OH-S) in area CA1 of hippocampal slices. The GABA$_B$ receptor agonist baclofen (BAC) hyperpolarizes pyramidal neurons by activating postsynaptic GABA$_B$ receptors, which mediate the late IPSP recorded from these neurons, and depresses synaptic potentials evoked by stimulation of Schaffer collateral/commissural fibers, presumably by activating presynaptic GABA$_B$ receptors. Low concentrations of 2-OH-S (50-100μM) antagonized the postsynaptic action of BAC and reduced the amplitude of the late IPSP. These concentrations of 2-OH-S did not block depression of EPSPs by BAC, however, higher concentrations (200-500μM) were effective. 200μM 2-OH-S shifted the dose-response curve for depression of EPSPs by BAC to the right by a factor of 4, indicating a pA$_2$ value of 4 for 2-OH-S at presynaptic GABA$_B$ receptors. 2-OH-S did not change excitatory transmission or passive membrane properties. Phaclofen (0.5-1mM) also blocked the late IPSP but had only a weak effect at presynaptic receptors. We conclude that 2-OH-S is a more potent antagonist than phaclofen at pre- and postsynaptic GABA$_B$ receptors in the hippocampus. 2-OH-S and phaclofen may have a higher affinity for postsynaptic than presynaptic GABA$_B$ receptors, supporting the idea that pre- and postsynaptic GABA$_B$ receptors can be distinguished pharmacologically. Supported by the ONR.

429.5

MECHANISMS OF BACLOFEN- AND OPIOID-INDUCED DISINHIBITION IN AREA CA1 OF RAT HIPPOCAMPUS N.A. Lambert, N.L. Harrison[1] and T.J. Teyler Department of Neurobiology, Northeastern Ohio Universities College of Medicine, Rootstown, OH 44272 and [1]Department of Anesthesia and Critical Care, The University of Chicago, Chicago, IL 60637.

We studied depression of inhibitory postsynaptic potentials (IPSPs) by the GABA-B receptor agonist (±)-baclofen and the μ opiate receptor agonist [D-Ala^2,N-Me-Phe^4,Gly-ol^5]-enkephalin (DAGO) using intracellular recording in the hippocampal slice preparation. Baclofen (0.1-10μM) and DAGO (0.5-5μM) reversibly depressed monosynaptic IPSPs (mIPSPs) recorded from pyramidal neurons in the presence of the excitatory amino acid receptor antagonists DNQX (25μM) and APV (40μM); adenosine (100μM) did not affect mIPSPs. The GABA-B receptor antagonist 2-hydroxy-saclofen (200μM) antagonized baclofen-induced depression of mIPSPs. Barium chloride (1.0-2.0mM), which blocks the K-current induced by baclofen and DAGO, prevented baclofen-induced hyperpolarization but not depression of mIPSPs by baclofen or DAGO. Baclofen and DAGO depressed IPSPs and mIPSPs when applied locally near the recording electrode, but were ineffective when applied near the stimulating electrode in stratum radiatum. These results suggest that baclofen and opioids disinhibit pyramidal neurons in area CA1 of the rat hippocampus by activating receptors located on the terminals of GABAergic neurons, and by a mechanism that is insensitive to barium. Supported by the ONR.

429.6

GABAb RECEPTOR BINDING IN SYNAPTIC MEMBRANES THAT HAVE (ADULT RAT CEREBRUM) OR LACK (HIPPOCAMPAL CULTURE) A POSTSYNAPTIC POTASSIUM CONDUCTANCE COUPLED TO GABAb RECEPTORS. M.I. Al-Dahan* and R.H. Thalmann, Baylor College of Medicine, Houston, TX 77030.

GABAb receptors in our cultures lack coupling with K channels (Hablitz and Thalmann, unpublished) that adult neurons possess in abundance. Do the receptors themselves differ? Membranes were prepared as described earlier (Al-Dahan and Thalmann, J. Neurochem. 1989, v53, p982) from adult rat, or from hippocampi dissociated at postnatal day 1 and cultured 7 days. Scatchard analysis of saturation studies of specific binding of (^{3}H)-baclofen indicated a single binding site in either preparation, with an affinity in cultured neurons (KD=45-60nM) similar to that in adult cerebrum (Kd=49+8nM). The ability of GABAb antagonists to displace baclofen binding was similar in both preparations: In both, the IC50 for phaclofen was approximately 100µM, for saclofen, 10µM. The regulation of binding by calcium and by GTPγs was also similar in that in either preparation binding was totally dependent upon Ca, and could be completely suppressed by GTPγs (100µM). Thus all the GABAb receptors in each membrane may be affected by Ca and coupled to G-proteins. Even though the coupling of receptors to K channels differs in the two preparations, we have thus far detected no differences in the receptors themselves. Supported by NIH grant NS21713.

429.7

Presynaptic and postsynaptic GABA$_B$ receptor pharmacology in spinal cord neurons. G. Kamatchi*and M.K. Ticku. Department of Pharmacology, University of Texas Health Science Center, San Antonio, TX 78284-7764.

Functional assays for measuring pre- and postsynaptic GABA$_B$ receptors were developed in primary cultured spinal cord neurons, using ^{86}Rb-fluxes. GABA$_B$ receptor activation with baclofen inhibited the ^{86}Rb-efflux induced by depolarization (100mM KCl), but not by calcium ionophore A23187, suggesting that GABA$_B$ receptor activation depresses the voltage-activated Ca^+-channels presynaptically. The tricyclic antidepressants, but not the MAO inhibitors, also inhibited the ^{86}Rb-efflux induced by both high potassium as well as A23187. The effect of baclofen, but not that of antidepressants was blocked by phaclofen and pertussis toxin (PTX). The GABA$_B$ receptor activation also produced an influx of ^{86}Rb. This effect of baclofen was concentration-dependent and inhibited by phaclofen and CGP35 349. Adenosine and 5-HT also produced ^{86}Rb-influx in these neurons. The effect of baclofen was not additive with either 5-HT or adenosine. Moreover, PTX pre-treatment blocked the hyperpolarization induced with these agents, indicating that these three receptors are linked to the same K^+-channel via the G-proteins.

429.8

GABA-B RECEPTORS MODULATE GLUTAMATE RELEASE IN THE RAT CAUDATE AND GLOBUS PALLIDUS. R. Singh, Dept. of Neurol. Sci. Rush Medical College, Chicago, IL 60612.

In continuation of our previous work (Neurosci. Abst. 15:583,235.14,1989), the role of GABA-B receptors in release of glutamate in caudate(CA) and globus pallidus (GP) was studied. Male rats (250-300g) were anaesthetized with halothane/O_2 and dialysis probes were implanted into CA or GP. Lactate-Ringer(pH-7.4) was perfused through the probes at 1ul/min. Dialysates were collected at 20 min intervals and analyzed for aminoacids by OPA derivatization and HPLC with fluorescence detection. After collection of 3 basal samples 100 uM baclofen was added to the perfusate for 40 min and sampling continued for 2h. Changes in the spontaneous release of glutamate, GABA and other amino acids were expressed as %change from the base line levels. Baclofen decreased glutamate release by 30±5 % in CA vs 39±7 % in GP. GABA release was decreased in CA by 23±3 % vs 32±5 % in GP. No other amino acid showed any change during baclofen perfusion. These results indicate significant modulation of glutamergic and GABAergic functions by GABA-B receptors in CA and GP nuclei of the basal ganglia.

EXCITATORY AMINO ACIDS: NMDA RECEPTOR GLYCINE AND POLYAMINE SITES

430.1

IN VIVO AND *IN VITRO* STEREOSELECTIVITY OF THE GLYCINE SITE OF THE N-METHYL-D-ASPARTATE RECEPTOR FOR THE (R)-ENANTIOMER OF HA-966 (1-HYDROXY-3-AMINO-PYRROLIDONE-2). L.M. Pullan, R.J. Stumpo,* M. Britt,* M.J. Chapdelaine,* R.A. Keith, D. LaMonte,* T.J. Mangano,* J. Patel, R.J. Powel,* P.J. Warwick,* W.C. Zinkand, and A.J. Salama. ICI Pharmaceuticals Group, ICI Americas, Wilmington, DE 19897

We report here the comparison of the *in vivo* stereoselectivity of the glycine receptor for the (R)-enantiomer of HA-966 (1-hydroxy-3-amino-pyrrolidone-2) to the stereoselectivity we have observed *in vitro*. The glycine antagonist site of the N-methyl-D-aspartate (NMDA) receptor *in vitro* shows stereoselectivity for the (R)-HA-966 in: [^{3}H]glycine binding in rat brain synaptosomal membranes, glycine stimulated [^{3}H]TCP (1-[1-(2-thienyl) cyclohexyl]piperidine) binding in rat brain synaptosomal membranes, prevention of NMDA induced cytotoxicity in cortical cultures, NMDA-evoked [3H]norepinephrine release in rat hippocampal slices, and glutamate-evoked contractions of guinea pig ileum. In each assay, the effects of (R)-HA-966 could be reversed by addition of glycine. We now confirm that the (R)-enantiomer is more potent *in vivo* against NMDA or D-serine evoked cGMP in cerebellum. Although the glycine receptor is stereoselective for (R)-HA-966, the (S)-enantiomer is not without activity in a number of assays and can confound the interpretation of results with racemic HA-966.

430.2

ANALYSIS OF ETHANOL (EtOH) INTERACTION WITH GLYCINE POTENTIATION OF NMDA-ACTIVATED ION CURRENT. G. White, D.M. Lovinger, R. W. Peoples, and F.F. Weight. Section of Electrophysiology, National Institute on Alcohol Abuse and Alcoholism, Rockville, MD 20852.

The amplitude of NMDA-activated ion current is reduced in the presence of intoxicating concentrations of EtOH (5-100mM). We have investigated whether the inhibition of NMDA-activated current by EtOH results from an alteration of the interaction between glycine and its binding site on the receptor/ionophore complex. Experiments were carried out on cultures of mouse hippocampal neurons using the whole-cell patch-clamp technique. We evaluated the effect of 50 mM EtOH on the amplitude of the NMDA-activated ion current at different added glycine concentrations. Inhibition by EtOH was 33±11, 33±2, 30 ±5, 28±4, and 18±1 at 10 nM, 100 nM, 500 nM, 1 µM, 10 µM glycine, respectively. The EC_{50} for glycine was similar in the absence and presence of EtOH (351 vs 360 nM, respectively). Reduction in EtOH inhibition was significant only at 10 µM glycine ($p \leq 0.01$, paired t-test), while maximal potentiation of the NMDA current was observed at 1 µM glycine. Thus, high concentrations of glycine reduced the effect of EtOH, whereas lower concentrations did not. Similar results were obtained in the presence of 10 µM strychnine. Finally, addition of 20 µM 7-chlorokynurenic acid to solutions with 10 µM glycine reduced the amplitude of NMDA-activated current to the amplitude in 100 nM glycine but did not reverse the effects of glycine on EtOH inhibition (19±2 vs 18±1%, $p>0.25$). These observations suggest that ethanol does not act in a competitive manner with nM concentrations of glycine, but that some interaction between EtOH and glycine can occur at µM concentrations of glycine.

430.3

AUTORADIOGRAPHIC EVIDENCE FOR DIFFERENTIAL COUPLING OF THE GLYCINE ANTAGONISTS ACBC AND 7-CHLOROKYNURENATE TO THE [3H] CPP RECOGNITION SITE. R.P. COMPTON, W.F. HOOD, J.B. MONAHAN, J.P. BIESTERFELDT*, G.E. FRIERDICH AND K.E. MILLER, CNS Diseases Research, G.D. Searle & Co., St. Louis, MO 63198

The results of this autoradiographic study of rat forebrain slices indicate that the glycine antagonists 1-aminocyclobutyl-1-carboxylate (ACBC) and 7-chlorokynurenate (7CHLKYN) differentially modulate interactions at the [3H]3-(2-carboxypiperazin-4-yl)propyl-1-phosphonate ([3H]CPP) recognition site. Scatchard analysis indicates that in the rat hippocampus, 50 uM ACBC induces an increase in the number of [3H]CPP binding sites (B_{max}: 0.46 +/- 0.01 to 0.74 +/- 0.01 pmoles in the stratum radiatum of CA1) without significantly altering the affinity. Although the B_{max} increases in the presence of ACBC, a linear Scatchard analysis along with a Hill number near unity suggests similar characteristics for both the basal sites and induced sites. These effects of ACBC on [3H]CPP binding are observed throughout the hippocampus. In contrast, 7-CHLKYN does not stimulate [3H]CPP binding in the hippocampus, specifically, stratum oriens and stratum radiatum of CA1 and CA3 as well as dentate molecular layer. More significantly, 7CHLKYN non-competitively inhibits the [3H]CPP binding enhancement induced by ACBC.

These findings suggest that ACBC and 7CHLKYN represent distinct classes of glycine antagonists acting at separate recognition sites, which differentially modulate interactions at the coupled NMDA antagonist recognition site labelled with [3H]CPP. The increase in [3H]CPP binding sites induced by ACBC may be a result of the unmasking of nascent sites or the conversion of NMDA agonist-preferring sites to antagonist-preferring sites.

430.4

INTERACTIONS BETWEEN GLYCINE AND THE *N*-METHYL-D-ASPARTATE RECEPTOR: KINETIC MECHANISMS R. Sircar M. J. Frusciante* and S.R. Zukin Departments of Psychiatry and Neuroscience, , Albert Einstein College of Medicine/Montefiore Medical Center, Bronx, New York.

Glycine has been shown to potentiate *N*-methyl-D-aspartate (NMDA) receptor-mediated responses via its interaction with a strychnine-insensitive glycine recognition site. Glycine binding sites and NMDA receptors have identical receptor distribution patterns in rat brain. Specific antagonists at this glycine site would be important pharmacological probes of the mechanisms of interaction of the glycine and NMDA recognition sites. The potent glycine receptor antagonist 7-chlorokynurenic acid (7Cl-KYN) dose-dependently inhibits [3H]MK-801 binding to the PCP receptor and this effect is reversed by the addition of glycine. Association of [3H]MK-801 to its binding site located within the NMDA receptor-associated ion channel, occurs in a biexponential fashion. The fast component of [3H]MK-801 binding serves as a marker of activated NMDA channels. Incubation with 7Cl-KYN completely abolished the fast component of [3H]MK-801 association in 4 out 5 experiments and in the one experiment where the fast component was detected, it accounted for less than half of that seen in its absence. 7Cl-KYN-induced inhibition of the fast component of [3H]MK-801 association was reversed by the addition of glycine. Since the fast component of [3H]MK-801 association represents ligand binding to the PCP receptor via the open NMDA channel, selective reduction of this component may be used as a valid marker for glycine antagonists.

Support: USPHS DA-03383, Ritter Foundation, David Berg Family Fund (SRZ), and generous support by the Department of Psychiatry, Dr. H.M. van Praag, Chairman (RS, SRZ)

430.5

INTERACTIONS BETWEEN POLYAMINES, GLYCINE AND GLUTAMATE AT THE *N*-METHYL-D-ASPARTATE RECEPTOR. I.Z. Nussenzveig*, R. Sircar, M.J. Frusciante*, D.C. Javitt, S.R. Zukin. Departments of Psychiatry and Neuroscience, Albert Einstein College of Medicine, Bronx, NY 10461

The goal of our study was to determine mechanisms involved in the interactions between spermidine and the NMDA receptor. The effect of increasing spermidine concentrations upon specific binding of [3H]MK-801 to well-washed, frozen-thawed synaptic membranes derived from rat forebrain was studied in the presence and absence of 7-chlorokynurenic acid (7-Cl KYNA) or D-(-)-2-amino-5-phosphono-valeric acid (D(-)AP5) with and without added L-glutamate and/or glycine. 7-Cl-KYNA attenuates NMDA receptor functioning by acting as a competitive antagonist of the NMDA receptor-associated glycine site, and D(-)AP5 as a competitive antagonist at the glutamate (NMDA) site.

7-Cl-KYNA attenuated [3H]MK-801 binding in the presence of spermidine alone or in the added presence of L-glutamate, but not in the presence of glycine. D(-)AP5 (1 μM) attenuated [3H]MK-801 binding in the presence of spermidine alone, but not in the presence of L-glutamate and/or glycine. D(-)AP5 (10 μM) attenuated [3H]MK-801 binding in presence of spermidine alone or with glycine, but not in the presence of L-glutamate.

Our results suggest: 1) Spermidine-induced stimulation of NMDA-receptor functioning requires the presence of agonist. 2) Spermidine-induced stimulation of NMDA-receptor functioning requires the presence of glycine, or alternatively, 7-Cl-KYNA may function as an inverse agonist at the glycine site.

Support: USPHS DA-03383, Ritter Foundation (SRZ); USPHS MH-00631 (DCJ); Dept. of Psychiatry, AECOM (Dr. H.M. van Praag, Chairman)

430.6

IFENPRODIL BLOCK OF NMDA CHANNELS IS PARTIALLY GLYCINE DEPENDENT. P.Legendre*†& G. Westbrook, †INSERM, Bordeaux, France and Vollum Institute, Oregon Health Sciences Univ., Portland, OR.

The vasodilator ifenprodil has recently been shown to antagonize NMDA receptors in several experimental preparations, but its mechanism of action remains unclear. It has been suggested that ifenprodil (IFL) antagonizes NMDA receptors by an interaction with polyamines (Carter et al., EJP 164, 611,1989), but Reynolds & Miller (Mol.Pharm 36,758,1989) have proposed that IFL stabilizes an inactivated form of the channel. We used whole-cell and single channel recording from cultured hippocampal neurons to test the action of IFL on NMDA channels.

Neurons from neonatal rats were dissociated and grown in cell culture for 1-2 weeks. For recording, the extracellular solution contained (mM): Na 165, K 2.5, Ca 1, Mg 0, HEPES 10, glucose 10; glycine (30 nM - 100 µM); picrotoxin, strychnine, and tetrodotoxin were also added. Patch pipette solutions contained (mM): Cs 140, EGTA 10, Mg 1, HEPES 10, Cl 126, Fl 14. Cation salts were ultrapure; NMDA (5 or 10 µM) and IFL (0.1 - 1000 µM) were applied by flowpipes.

In 500 nM glycine, maximal concentrations of IFL blocked 95% of whole cell NMDA currents. The block by 100 µM IFL was partially overcome by increasing glycine to 100 µM. IFL appeared to have a biphasic inhibition curve with approximate IC50s of 1.3 and 240 µM. The high affinity component blocked ≈ 35% of the NMDA current. IFL block was not voltage- or use-dependent. The half-recovery from IFL block was 16 sec and was not increased by continous presence of agonist or by holding at positive membrane potentials. On outside-out patches, IFL reduced both the number and mean open time of NMDA channels with no effect on single channel conductance. IFL (5 µM) blocked 70% of channel activity (n=5) which was much greater than expected from IFL inhibition of the macroscopic current, perhaps suggesting that intracellular dialysis alters NMDA channel sensitivity to IFL. Supported by INSERM, NATO Fdn., NIH and the McKnight Foundation.

430.7

IFENPRODIL INHIBITION OF [3H]GLYCINE BINDING TO THE NMDA RECEPTOR: INTERACTIONS WITH POLYAMINES. R.W. Ransom. Merck Sharp & Dohme Research Laboratories, West Point, PA 19486.

Ifenprodil has been shown to be a relatively potent noncompetitive inhibitor of NMDA receptor activity. It has been proposed that this antagonism is due to an interaction with the polyamine recognition site associated with the receptor complex. In the present study we have addressed this question by examining the interactions of spermine and ifenprodil at the NMDA receptor's glycine modulatory site. Using a thoroughly washed rat brain membrane preparation, ifenprodil partially inhibited [3H]glycine binding with an $IC_{50} \approx 35$ nM). The maximal level of inhibition was 39% of control binding under basal conditions. The addition of 100 µM L-glutamate stimulated binding by 40% and had no effect on the IC_{50} value for ifenprodil. However, ifenprodil could not completely reverse the stimulation of [3H]glycine produced by L-glutamate. Spermine maximally enhanced binding by 350% with an EC_{50} = 14 µM. Concentrations of spermine that enhanced [3H]glycine binding produced a dose-dependent increase in the IC_{50} value for ifenprodil inhibition of binding. At 1 mM spermine, ifenprodil blocked [3H]glycine binding with an $IC_{50} \approx 1.5$ µM. Yet, high concentrations of ifenprodil were unable to completely reverse the stimulation of binding produced by the polyamine. In related experiments, ifenprodil was found to reduce the maximum level of binding enhancement produced by spermine. These results indicate that spermine and ifenprodil do not competitively interact with respect to their effects on the NMDA receptor's glycine recognition site.

430.8

DIFFERENCES BETWEEN ANTAGONISM OF NMDA-INDUCED ACUTE EXCITOTOXICITY BY IFENPRODIL AND OTHER NMDA ANTAGONISTS G.D. Zeevalk and W.J. Nicklas Dept. of Neurology, UMDNJ-RWJ Med. Sch., Piscataway, NJ 08854

Acute excitotoxicity in embryonic day 13 chick retina induced by incubating with 50µM NMDA for 30min is demonstrated histologically by swelling of certain populations of neurons and by release of GABA. These effects are completely inhibited by noncompetitive antagonists, (+)MK-801 (IC_{50},0.02µM); (-)MK-801 (IC_{50},0.28µM) or by the competitive antagonist CGS 19755 (IC_{50},0.62µM). Ifenprodil (IFEN) also antagonizes NMDA-induced GABA release (IC_{50},1.26µM) however, it differs from other NMDA antagonists in 2 respects. Firstly, while other antagonists completely protect all NMDA sensitive neurons, IFEN fails to protect a select subpopulation of neurons. Secondly, studies done to determine temporal efficacy of antagonism demonstrate that MK-801 and CGS 19755 can protect when added after NMDA, the amount of protection being inversely related to the time of addition of antagonist, while IFEN is only protective when added prior to NMDA addition. This suggests that IFEN can only interact with the NMDA receptor when it is in an "inactive" state. To determine if the unprotected neurons seen in the presence of IFEN and NMDA represent neurons whose NMDA receptors are currently "active", retina was incubated with either TTX (2µg/ml), muscimol (0.5mM) or CGS 19755 (5 µM) prior to or during incubation with IFEN (10µM) plus NMDA (50µM). Such treatment did not alter the response in the IFEN-insensitive, NMDA-sensitive population suggesting that in addition to IFEN's differential action at "active" versus "inactive" NMDA receptors, IFEN may unmask a heterogeneity in the NMDA receptor population.

430.9

SPERMINE DEPOLARIZES THE RAT CORTICAL WEDGE AND POSITIVELY MODULATES SPONTANEOUS EPILEPTIFORM DISCHARGES. L.J. Robichaud and P.A. Boxer, Parke-Davis Pharm. Res. Div., Warner-Lambert Co., Ann Arbor, MI 48105.

The polyamine spermine reportedly modulates binding of MK-801 and glycine to allosteric receptor sites of the NMDA receptor-ion channel complex. We examined functional responses in the rat cortical wedge preparation to spermine in Mg++ free buffer with TTX (0.5 μM). Spermine (100 μM-3 mM) induced slow dose-dependent repeatable depolarizations which were not blocked by Mg++, the NMDA antagonist CPP, the AMPA/kainate/glycine antagonist DNQX, nor ifenprodil (a reported ligand of both the polyamine and sigma receptor sites). In the absence of TTX, spermine (100 μM-1 mM) increased (57-178%) the rate of spontaneous epileptiform discharges (SED) in a dose- and time-dependent manner. Ifenprodil (30 μM) inhibited SED; neither spermine (100 μM-3 mM) nor glycine (1 mM) reversed this inhibition. The ornithine decarboxylase inhibitor, DFMO (MDL 71782A) (100 μM, 2 hrs) did not inhibit SED. In conclusion, spermine showed functional responses in the rat cortical wedge model in both the presence and absence of TTX, with unclear mechanisms of action. This study did not indicate a competitive interaction between spermine and ifenprodil in direct depolarizations or SED. Activation of NMDA, AMPA, kainate, or glycine recognition sites do not appear to be involved in spermine-induced depolarizations.

430.10

POLYAMINE ANTAGONIST REDUCES NMDA-INDUCED DEPOLARIZATION, BUT ALSO REDUCES MAGNESIUM ANTAGONISM. M.A. Bowe and J.V. Nadler. Depts. Pharmacology and Neurobiology, Duke Univ. Med. Ctr., Durham, NC 27710.

We have demonstrated that Mg^{2+} less potently antagonizes depolarizations evoked by NMDA in 10-15 day old rats than in adults. Endogenous polyamines regulate opening of the NMDA channel, and they are most abundant during development. The polyamine 1,10-diaminodecane (DA10) inhibits the enhancement of [^{3}H]MK-801 binding produced by glutamate, glycine and spermine. We therefore used DA10 as a tool to determine whether polyamines regulate the sensitivity of the NMDA receptor to Mg^{2+}. A grease-gap preparation was used to study depolarizing responses of CA1 hippocampal pyramidal cells to NMDA.

In the absence of added Mg^{2+}, DA10 (316 μM) noncompetitively antagonized NMDA-evoked depolarizations, but did not affect depolarizations evoked by AMPA. The effect of DA10 was significantly greater in preparations from adult animals than in preparations from 10-15 day old animals. This difference may be explained by the greater concentration of polyamine present in younger animals. DA10 also greatly reduced the effectiveness of 1 mM Mg^{2+} in antagonizing NMDA-evoked depolarizations. The effect of Mg^{2+} was reduced by 70% in preparations from adult animals and was virtually abolished in preparations from 10-15 day old rats.

These results suggest that polyamines regulate not only the sensitivity of the NMDA receptor to agonist, but also its sensitivity to Mg^{2+}. DA10 may act as a polyamine antagonist for the former action and as a polyamine agonist for the latter action. (Supported by NIH grant NS 16064.)

430.11

BINDING OF [^{125}I]IFENPRODIL TO THE POLYAMINE-SENSITIVE DOMAIN OF THE NMDA RECEPTOR. P.M. Beart, L.D. Mercer*, A.J. Searle* and B. Jarrott*, University of Melbourne, Clinical Pharmacology, Austin Hospital, Heidelberg, 3084, Australia.

The NMDA receptor-ionophore complex consists of a number of interacting domains, including one for polyamines where ifenprodil binds to exert its non-competitive antagonism. Ifenprodil was iodinated with Na^{125}I/chloramine-T, purified and its binding characterized. In cortical membranes, binding of [^{125}I]ifenprodil was saturable (K_d 145nM, B_{max} 2.5 pmol/mg protein) and to a single site. Specific binding was reversible, being dissociable with 1mM spermine and poorly dissociable with 16.7μM SL 82.0715, and represented 70-75% of total binding by filtration. Binding was displaced by polyamines and drugs; rank order of potency SL 82.0715 = tibalosine > nylidrin = isoxsuprine. Inhibition constants for spermine and spermidine were 15 and 44μM, respectively. GBR 12909 (3μM) failed to affect the potency of SL 82.0715, tibalosine and spermine. Binding to synaptic membranes (K_d 150nM) was stimulated by NMDA (100%) and glycine (85%). In autoradiographic studies using slide-mounted sections, binding sites for [^{125}I]ifenprodil were localized in several regions including anterior cingulate cortex, hippocampus and amygdala. Whilst the polyamine site of the NMDA receptor can be successfully labelled with [^{3}H]ifenprodil (Shoemaker et al., Eur. J. Pharmacol. 176, 249, 1990), the [^{125}]ligand is a rapid, convenient and inexpensive alternative.

430.12

NMDA RECEPTOR BINDING PROPERTIES OF 5,7-DICHLORO-KYNURENIC ACID, A RADIOLABELED GLYCINE SITE ANTAGONIST. B.M. Baron, A.L. Slone*, B.W. Siegel*, B.L. Harrison*, S.D. Hurt*, M.W. Dudley, and M.G. Palfreyman. Merrell Dow Research Institute, Cincinnati, OH 45215, DuPont NEN, Boston, MA 02118.

The kynurenic acid (KA) derivative, 5,7-dichlorokynurenic acid (5,7-DCKA) is a potent and selective inhibitor of strychnine-insensitive [^{3}H]glycine binding to rat brain membranes (IC_{50} = 79 nM) whereas IC_{50} values were >100 μM at a variety of other glutamate-related and non-glutamate neurotransmitter receptors. 5,7-DCKA non-competitively antagonized the NMDA-mediated elevation of cGMP content of neonatal rat cerebellar slices. This antagonism was prevented by glycine or D-serine.

Using methods described for [^{3}H]glycine binding, we found that [^{3}H]5,7-DCKA (17.6 Ci/mmol) bound to rat brain membranes with K_d = 69 nM and B_{max} = 14.5 pmol/mg protein. A concentration of 10 nM [^{3}H]5,7-DCKA gave approximately 9,000 dpm (total) and 3,000 dpm (non-specific) and exhibited a pharmacological profile which was consistent with labeling of the strychnine-insensitive glycine binding site. IC_{50} values (μM) were: 5,7-DCKA (0.03), glycine (0.3), D-serine (0.6), 7-Cl-KA (0.5), KA (10), L-serine (27), HA-966 (49), indole-2-COOH (89). Binding was allosterically regulated by the glutamate recognition site; increased by NMDA, Asp, and Glu and inhibited by phosphonoamino acid antagonists. This ligand should be a valuable tool to probe the NMDA receptor complex.

430.13

KYNURENIC ACID ANALOGUES AS POTENT AND SELECTIVE ANTAGONISTS AT THE GLYCINE SITE ON THE NMDA RECEPTOR. A.C. Foster, J.A. Kemp, P.D. Leeson, S. Grimwood, G.R. Marshall, T. Priestley, R.W. Carling and K.W. Moore Merck, Sharp and Dohme Res. Lab., Neurosci. Res. Ctr., Harlow, Essex, U.K.

The N-methyl-D-aspartate (NMDA) receptor possesses two separate amino acid recognition sites, one for acidic amino acids such as L-glutamate and NMDA and one for neutral amino acids such as glycine. The broad spectrum excitatory amino acid antagonist kynurenic acid (KYNA) has affinity for both sites, however substitution of Cl in the 7-position yields a compound (7-ClKYNA) with improved potency and selectivity as a glycine site antagonist (Kemp et al, Proc Natl Acad Sci, 85:6547, 1988). In a [^{3}H]glycine binding assay using P_2 membranes from rat cortex and hippocampus, the affinity of 7-ClKYNA (IC_{50} = 0.56 μM) was improved in the analogues 5,7-diClKYNA (IC50 = 0.2 μM) and 5-I,7-ClKYNA (IC50 = 0.032 μM). These new KYNA analogues retained good selectivity (> 2 orders of magnitude) versus other acidic amino acid binding sites.

In a rat cortical slice assay and whole cell voltage-clamp recordings from rat cultured cortical neurones, these compounds were selective, non-competitive antagonists of NMDA responses (respective Kb's of 3.0 and 0.41 μM in the cortical slice). Both compounds caused a complete flattening of the NMDA concentration-response curve, and their antagonism was reversed by co-application of glycine or D-serine, consistent with a selective action at the glycine site. The improved selectivity and affinity of these compounds for the glycine site on the NMDA receptor should make them useful as tools for further studies.

431.1

Opioid peptide modulation of dopamine release: An *in vivo* electrochemical study. R. J. W. Pentney and A. Gratton, Douglas Hosp. Res. Ctr, McGill Univ., Montréal, Canada, H4H 1R3.

The present study used high-speed chronoamperometry to examine the effects of locally applied DPDPE and DAGO, two selective delta and mu2 receptor agonists repectively, on basal and K+-evoked release of dopamine (DA) in striatum (CPU) and nuc. accumbens (NAcc). Male rats were anesthetized with either chloral hydrate or urethane, tracheotomized and placed in a stereotaxic frame. Nafion-coated carbon fiber electrochemical electrodes were cemented to double-barrel micropipette assemblies (o.d. 30-40 um) with a tip separation of 200-300 um and lowered to various CPU and NAcc sites. One barrel contained a KCl solution while the other barrel contained either DPDPE or DAGO (1,10 or 100 nM). Electrochemical recordings were obtained by applying to the electrochemical electrode, at a rate of 5 Hz, a +0.5V oxidation potential relative to a Ag/AgCl reference electrode. The reduction current was also monitored to confirm the dopaminergic nature of the electrochemical signals. In both CPU and NAcc, local pressure microejection of DPDPE had no effect on basal levels of DA but significantly affected the depolarization-evoked release of DA caused by local K+ application. However, the effects of DPDPE on stimulated DA release depended on the anesthetic used. In urethane anesthetized animals, 1nM DPDPE caused a suppression of DA release, but mostly potentiated DA release when chloral hydrate was used. Both the suppressive and potentiating effects of DPDPE tended to be greater in CPU than in NAcc and could be partially blocked by naloxone (2 mg/kg, i.p.). DAGO produced inconsistent results; potentiating DA release on a few occasions but generally having no effect. Finally, locally applied morphine, another mu agonist, caused large increases in basal signals. However, these effects were discounted, since morphine itself was found to be electroactive. In conclusion, the present data indicate that enkephalins modulate the release of DA at the terminal via a delta receptor. The inconsistent effects found with DAGO may reflect the patchy distribution of mu2 receptors in these areas. However, these data must be interpreted cautiously in light of the interactions of these drugs with the anesthetics used. Funded by NSERC Canada.

431.2

ELECTROPHYSIOLOGICAL STUDIES OF MIDBRAIN DOPAMINE NEURONS IN CULTURE. D.L. Cardozo Department of Neurobiology, and Program in Neuroscience, Harvard Medical School, Boston, MA 02115.

I have removed midbrain dopamine (DA) neurons from neonatal rats and routinely maintained them in dissociated cell culture for several months. The DA phenotype has been confirmed by catecholamine fluorescence and by tyrosine hydroxylase immunocytochemistry. Living DA neurons have been identified for electrophysiology by 5,7-DHT fluorescence, and recordings made with both high resistance and patch electrodes. The observed physiological properties of cultured DA neurons are consistent with results reported from *in vivo* and slice preparations. These include long-duration action potentials (2.5-4 ms); 1-5 Hz pacemaker-like firing activity; 450 ms slow depolarization to action potential; and anomalous rectification. In addition, DA cell firing was inhibited by applied DA or (+/-)-PPHT, a potent D2 agonist. These results suggest that this culture system will be useful for acute and chronic pharmacological studies of DA neurons. (Supported by funds from the Commonwealth Res. Ctr.,MA Mental Health Ctr;DA 04582; Scottish Rite; and NSCERC, Canada.)

431.3

REPEATED ADMINISTRATION OF D1 DOPAMINE RECEPTOR ANTAGONISTS FAILS TO INACTIVATE DOPAMINE NEURONS. S.R. Wachtel and F.J. White. Wayne State Univ., Sch. of Med., Dept. Psychiatry, CCN Program, Neuropsychopharmacology Lab, Lafayette Clinic, Detroit, MI 48207

Based on the findings that D1 dopamine (DA) receptor antagonists can block the behavioral effects of both D1 and D2 receptor agonists, it has been suggested that D1 antagonists may be effective antipsychotic drugs (APDs). Moreover, a variety of pharmacological studies have led to predictions that D1 DA receptor antagonists may have a clinical profile characteristic of atypical APDs such as clozapine. One electrophysiological model of atypical APDs action is the ability of repeated administration of an APD to decrease the number of spontaneously active DA neurons in the ventral tegmental area (A10), but not in the substantia nigra (A9), by the process of depolarization inactivation (DP block). In the present study, the effect of repeated administration of the D1 antagonists SCH 23390 and SCH 39166 on the activity of both A9 and A10 DA neurons was investigated in the rat. In contrast to the selective decrease in the number of spontaneously active DA neurons in A10 observed after continuous administration of the D1 antagonist SCH 23390 (0.29 mg/kg/day, s.c.) via mini osmotic pumps, repeated administration of SCH 23390 or SCH 39166 (0.5/mg/kg, s.c., b.i.d.) failed to alter the activity of DA neurons in either A9 or A10. Although the effects of continuous and repeated administration of SCH 23390 appear to differ, SCH 23390 produced relatively small decreases (< 30%) in DA neuronal activity with both administration regimens as compared to other APDs such as haloperidol (> 50%). In addition, the decrease observed after continuous administration of SCH 23390, in contrast to haloperidol, did not appear to be due to DP block as it was not reversed by membrane hyperpolarization with apomorphine (0.01 or 0.05 mg/kg, i.v.). In conclusion, these results indicate that D1 DA receptor antagonists do not exert electrophysiological effects on DA neurons characteristic of standard APDs, and suggests that either D1 antagonists might not be APDs or that DP block may not be a necessary component of APD action. (Supported by MH-40832, DA-04093 and the Michigan Departmet of Mental Health.)

431.4

HA-966 INHIBITS THE ACTIVITY OF MESENCEPHALIC DOPAMINE (DA)-CONTAINING NEURONS THROUGH A NON-NMDA RECEPTOR-MEDIATED MECHANISM. P.D. Shepard, H. Lehmann-Romeyn*, J.H. Kogan, R.H. Roth, and B.S. Bunney. *Depts. of Psychiatry and Pharmacol., Yale Univ. Sch. of Med., New Haven CT. 06510 and Maryland Psychiatric Research Cntr., Baltimore, MD 21228.*

HA-966 (+ 1-hydroxy-3-amino-pyrrolidinone-2) has been shown to suppress the activity of mesencephalic DA-containing neurons *in vivo*. Structurally related to the cyclic anhydric form of GABA, this compound is believed to interact with central GABAergic receptors. Recently, however, HA-966 has also been shown to antagonize glycine at its allosteric site on the NMDA receptor (Foster and Kemp, J. Neurosci., 9:2191) - an effect which appears to be mediated solely by the (+) enantiomer (Singh et. al., PNAS, 87:347). In the present series of experiments, we sought to determine the extent of the contribution made by GABA and NMDA receptor subtypes to the effects of HA-966 on nigral DA neurons recorded *in vivo* and *in vitro*. Administration of a bolus dose of the racemic form of HA-966 (10-40 mg/kg, i.v.) inhibited the firing rate of all cells tested (n=20) in chloral hydrate-anesthetized rats. In most cells (n=13), these changes were preceded by a transient increase in activity (10-40%). Doses exceeding 20 mg/kg caused a complete cessation of spontaneous firing. Both the initial rate of decline in firing rate (time required to inhibit activity by 50%) and latency to blockade of neuronal activity were dose-dependent. In contrast to the effects of the racemic form of the drug, systemic administration of the (+) enantiomer (40mg/kg) had no effect on the activity of nigral DA neurons. The effects of HA-966 (1 mM) on DA neurons studied in brain slices were qualitatively similar to those observed *in vivo*, with the exception of the early excitatory component, which was never observed (n=8). Bath application of bicuculline (50 uM) resulted in a modest increase in basal firing rate but failed to antagonize the inhibitory effects of HA-966. These data, together with our *in vivo* findings, indicate that the ability of HA-966 to inhibit the firing of nigral DA-containing neurons may not involve either $GABA_A$ or NMDA receptor-mediated mechanisms. Supported by NARSAD and PHS MH-28849 and 14092.

431.5

DOPAMINERGIC NEUROTRANSMISSION ASSESSED BY IN VIVO DIALYSIS IN THE VENTROLATERAL STRIATUM OF RATS FOLLOWING SYSTEMIC ADMINISTRATION OF SPECIFIC DOPAMINE (DA) AGONISTS AND ANTAGONISTS. Ronald E. See, Dept. of Psychology, Washington State University, Pullman, WA, 99164-4820.

Previous studies in rodents suggest that the ventrolateral striatum (VLS) is preferentially involved in the control of certain motor activities, particularly orofacial movements. The present study examined the effects of DA D1 and D2 receptor agonists and antagonists on DA release and metabolism in the VLS using in vivo microdialysis techniques. Female, Sprague-Dawley rats were anesthetized with Equithesin and unilateral guide cannulae implanted (A +0.2, L +3.5, V -5.0). Following one week of recovery, microdialysis probes with 3 mm of exposed dialysis membrane were inserted into the guide cannulae and perfusion with Ringer's solution initiated. Samples were collected every 20 minutes before and after drug injection. The following drugs and doses (mg/kg) were administered IP: the D2 antagonist, raclopride (0.5, 2.0, 4.0), the D2 agonist, quinpirole (0.03, 0.1, 0.3), the D1 antagonist, SCH 23390 (0.01, 0.05, 0.25), and the D1 agonist, SKF 38393 (0.3, 1.0, 3.0). Doses were selected based on previous studies which indicated significant behavioral effects at these doses. Perfusates were directly injected onto an HPLC column and analyzed for DA, DOPAC, and HVA using electrochemical detection. Raclopride markedly increased levels of DA, DOPAC, and HVA while quinpirole produced a decrease in all three. SCH23390 also stimulated the release of DA and increased metabolite levels but to a much lesser degree than raclopride. SKF 38393 failed to alter DA release and metabolism. These results suggest that the in vivo release of DA may be related more to D2 than D1 receptors.

431.6

IN VITRO EXTRA- AND INTRACELLULAR RECORDING FROM SINGLE MID-BRAIN DOPAMINE (DA) NEURONS WITH IDENTIFICATION OF THEIR PROJECTION SITES. T.H. Lee* and E.H. Ellinwood, C.M. Kuhn, and G. Einstein, Dept. of Psychiatry, Pharmacology and Neurobiology, Duke Univ. Med Ctr., Durham, NC 27710

We have recently initiated experiments to examine the differential regulatory mechanisms of single identified DA neurons in slice preparations. Since the various DA projection systems may each play a different role in the mediation of both normal and abnormal behaviors, we pre-labelled midbrain DA neurons by injecting rhodamine-labelled flourescent microspheres (RFM) into their projection sites.

RFM were injected into either the dorsolateral caudate or nucleus accumbens 2-4 days prior to extracellular single-unit recording. After a survival sufficient for retrograde transport of the RFM, 350 um slices of tissue were cut. Under epifluorescent illumination, neurons labelled with the microspheres could be clearly seen in the top layer of the slices as a tightly packed group. These surface neurons (top 80 um) did not exhibit spontaneous activity. However, those just below the surface neurons did. On the assumpton that the majority of these deeper neurons were RFM labelled, we recorded from them and found numerous spontaneously active DA neurons exhibiting pacemaker-like baseline activity and reversible inhibition by DA and GABAergic agonists. We are currently in the process of implementing intracellular recording and dye injection in order to: (1) examine the viability of the inactive surface DA neurons and (2) identify the projection area of the recorded neuron by the presence of double label. Results from these studies as well as the differential effects of DA and GABAergic agents on identified DA neurons will be presented.

431.7

***IN VIVO* MEASUREMENTS OF EXTRACELLULAR DOPAMINE AND NEUROTENSIN IN THE DORSOLATERAL AND MEDIAL PREFRONTAL CORTEX, PREMOTOR CORTEX, AND CAUDATE OF THE RHESUS MONKEY: EFFECT OF AMPHETAMINE.** B. Moghaddam, C.W. Berridge, A.J. Bean, B.S. Bunney, P.S. Goldmann-Rakic and R.H. Roth. Depts. Psych. & Pharm., Sec. Neuroanat. Yale Univ.Sch. Med., New Haven, CT 06510.

Microdialysis was utilized to assess the extracellular concentration of dopamine (DA) in the anesthetized Rhesus monkey. Concentric probes were placed in the caudate nucleus, premotor cortex and dorsolateral and medial prefrontal cortices. Basal DA levels were consistently detected in the caudate (1.9±1.1 fmoles/µl, perfusion rate = 1 µl/min) and premotor cortex (0.63±0.2 fmoles/µl). In prefrontal cortex (both medial and dorsolateral), DA was reliably detected in two out of three animals measuring approximately 0.3 fmoles/ul. Intravenous administration of amphetamine (1 mg/kg) enhanced extracellular DA levels in caudate by more than 36 fold (3651%±311). This effect is much greater than the 300-500% increase we observe in the rodent. Smaller increases (300%-800%) after amphetamine were detected in cortical areas of the primate. These studies demonstrate that basal extracellular DA can be measured in caudate and cortex of nonhuman primates and that DA levels are responsive to pharmacological manipulation. Preliminary studies showed no change in neurotensin levels in cortex and caudate following amphetamine. Supported by MH-44866.

431.8

SIMULTANEOUS MICRODIALYSIS MEASUREMENT OF EXTRACELLULAR DOPAMINE AND SEROTONIN IN THE PREFRONTAL CORTEX AND STRIATUM OF THE FREELY MOVING RAT. A.M. Rasmusson, L.E. Goldstein, B.S. Bunney, R.H. Roth. Dept. of Pharm. & Psych., Yale Univ.Sch. Med., New Haven, CT 06510.

Postsynaptically active doses of the serotonergic 5HT1a agonist, 8-OH-DPAT, selectively increase dopamine (DA) turnover in the prefrontal cortex (PFC) of the rat. We have further investigated this phenomenon using *in vivo* microdialysis in the freely moving rat. Single rats were stereotaxically implanted with striatal (CP) and bilateral PFC microdialysis probes, as well as with intraperitoneal catheters 18-24 hrs before experimentation. Extracellular DA and 5HT were monitored following normal saline and subsequent 8-OH-DPAT (225 ug/kg) injections. At a perfusion rate of 1.5 µl/min, basal DA and 5HT levels in the PFC of the freely moving rat were 0.09 and 0.14 fmol/µl, respectively, uncorrected for probe recovery; in the CP, 0.86 fmol/µl and 0.27 fmol/µl, respectively. After 8-OH-DPAT, DA in the PFC increased by approx. 200%; it remained stable in the CP. Maximal DA increases and 5HT decreases occurred within 1 hr of 8-OH-DPAT. DA and 5HT returned to baseline 2-3 hrs after treatment. These findings are consistent with differential regulation of the mesotelencephalic DA systems by the 5HT system. Supported by PHS T32 DK 07476, Tourette Syndrome Assoc., PHS T32 GM07205 and MH-14092.

431.9

THE INVOLVEMENT OF SUBTHALAMIC NUCLEUS PROJECTIONS TO THE SUBSTANTIA NIGRA IN THE REGULATION OF DOPAMINE NEURON BURST FIRING . I.D. Smith and A.A. Grace, *Departments of Behavioral Neuroscience and Psychiatry, University of Pittsburgh, Pittsburgh, Pennsylvania, 15260, USA.*

Substantia nigra (SN) dopamine (DA) neurons recorded *in vivo* fire in one of two patterns: 1) an irregular, single-spiking pattern or 2) burst firing. DA neurons will enter a burst firing mode when the DA system is compromised, such as following extensive striatal DA depletions or in response to the administration of DA receptor blockers. In contrast, DA neurons recorded *in vitro* in brain slices, in which much of their afferent input is sectioned, fire only in a very regular pacemaker pattern, with no evidence of burst activity. Evidence is presented here suggesting that burst firing in a sub-poulation of DA neurons is regulated by excitatory afferents from the subthalamic nucleus (STN).

In normal controls, 44.5% of DA cells exhibited burst firing (n= 28 rats). Burst firing could be induced by STN stimulation in the majority of cells recorded, with a progressive increase in the percentage of bursting cells occurring between 30 to 60 minutes following 1 minute of stimulation at 20 Hz (72.4%, n=5). After hemisection of the brain between the STN and the SN, averaged burst firing within the entire SN was unaltered (38.8%, n=10). However, there was a reduction in burst firing in cells recorded in the lateral (L) SN, as compared to centrally (C) and medially (M) located cells (LSN: 23.1% vs. control: 38.6%; CSN: 46.7% vs. control: 43.6%; MSN: 42.8% vs control: 50.4%). In each case, a number of lateral DA neurons were observed to fire in a very regular, pacemaker pattern, in some cases despite elevated discharge frequencies (eg. > 8 Hz). This is in marked contrast to the intact animal where pacemaker firing of DA cells, particularly at these high discharge rates, has not been observed. If the transections were placed anterior to the STN, little change was noted in DA cell firing pattern (44.8%, n=6). Regularization of lateral SN DA neuron firing and a decrease in the number of cells bursting (LSN: 7.1%, CSN: 36.0%, MSN: 43.8% n=9) was also produced by electrolytic lesions of the STN.

These data suggest that an excitatory projection from the STN modulates the firing pattern of lateral SN DA neurons. This pathway may be involved in the feedback increase in DA cell burst firing following reductions in striatal DA transmission secondary to disinhibition of the STN. (Supported by USPHS NS19608, MH42217, MH09660 and MRC of Canada)

431.10

EFFECTS OF STRIATONIGRAL PATHWAY HEMITRANSECTION ON FIRING PROPERTIES AND PHARMACOLOGICAL RESPONSES OF SUBSTANTIA NIGRA DOPAMINE NEURONS IN THE RAT. M.L. Pucak & A.A. Grace. *Depts. of Behav. Neurosci. & Psychiatry, U. of Pittsburgh, Pittsburgh, PA, 15260.*

The GABAergic striatonigral projection has been suggested to modulate the firing properties and the pharmacological responses of dopamine (DA) neurons in the SNzc. However, its functional importance remains unclear, since kainic acid lesion of the striatum does not alter DA neuron firing rate or response to apomorphine (APO). We have examined alterations in DA neuron electrophysiology after hemitransections of the striatonigral pathway using single unit extracellular recordings from DA neurons of rats. Pharmacological manipulation consisted of i.v. administration of APO in log dose fashion, followed by an equal dose of haloperidol (HAL). In a subset of rats, hemitransections of the striatonigral pathway were performed by making cuts with a glass knife at approximately A4.8. Data are presented as mean±SD. Hemitransection did not alter DA neuron firing rate (control= 5.0±2.3 (n=33), transected=4.4±2.1 (n=37) spikes/sec). Burst-firing was likewise unaffected; the % of neurons defined as bursting (controls=33.3, transected=20.0), the % of spikes fired as bursts (control=5.5±6.1, transected= 7.9±17.1), and the spikes per burst (control=2.5±7.3, transected=2.5±10.0) were unchanged (control, n=9; transected, n=10). The number of spontaneously active DA neurons encountered per electrode track also was unaltered (control=1.0±.1 (n=4), transected=1.3±.2 (n=3)). The ED_{50} for APO was not affected (control=7.7±5.7 (n=12), transected=6.7±6.2 (n=10) µg/kg). However, there was a change in the shape of the dose-response curve. In addition, a subset of DA neurons in the transected rats demonstrated a slowed return to baseline after administration of HAL. In such cases, administration of additional HAL was necessary to return firing rate toward baseline; this was never necessary when recording from controls. These data demonstrate that the striatonigral projection does not exert clear effects on the baseline firing rate or pattern of DA neurons, but may have subtle effects on their responses to pharmacological manipulation. (Supported by MH 09873, NS 19608, MH 42217).

431.11

MORPHOLOGICAL DEVELOPMENT OF THE SUBSTANTIA NIGRA IN THE POSTNATAL RAT J.M. Tepper, F. Trent and J.S. Rankin* Center for Molecular and Behavioral Neuroscience and Department of Biological Sciences , Rutgers University, Newark, NJ, 07102.

Recent studies indicate that the physiological and pharmacological properties of rat substantia nigra dopamine (DA) neurons undergo significant changes during the first four weeks of life. The present studies were carried out to examine the morphological correlates of the developmental changes in the neurophysiological properties of nigral DA neurons.

Sprague-Dawley rat pups of age postnatal day 1 (PD1), PD7, PD14, PD21, PD28 and adult rats were anesthetized and perfused with a saline wash followed by 4% paraformaldehyde and 0.2% glutaraldehyde. Brains were sectioned at 50-75 µm and processed for tyrosine hydroxylase (TH) immunocytochemistry by conventional means. The distribution of TH+ neurons and dendrites in substantia nigra was noted, and measurements of TH+ somatic sizes, and the diameters of proximal (25 µm from the soma) and distal (>400 µm from the soma) dendrites were made using a Quantex QX-7 image analysis system.

At PD1, pars compacta and pars reticulata were not clearly delineated; TH+ neurons and a dense plexus of fibers were scattered throughout the substantia nigra. By PD7 the density of TH+ neurons increased dorsally and decreased ventrally, and by PD14, a DAergic pars compacta and a non-DAergic pars reticulata could be clearly distinguished. At PD21, the density of TH+ neurons and dendrites in pars reticulata was further reduced, and by PD28 substantia nigra cytoarchitecture appeared as it does in the adult. TH+ somatic size increased significantly from PD1 (15.1±0.4 µm x 10.4±0.2 µm) to PD14 (25.3±0.5 µm x 14.9±0.3 µm; F=47.8, df=5, 283, p<.001), as did the diameter of the proximal (2.16±0.1 µm , PD1; 3.06±0.1 µm, PD14; F=12.83, df=5, 283, p<.001) and distal dendrites (0.62 ±0.01 µm, PD1; 0.69 +0.01 µm, PD14). Distal dendritic diameter peaked at PD21 (0.72+0.01 µm), decreasing to 0.61±0.01 µm at PD28. Neither somatic size nor proximal dendritic caliber changed between PD14 and adulthood.

These data demonstrate significant postnatal changes in the cytoarchitecture of the rat substantia nigra. The sizes and distribution of DAergic neurons and processes first resembled those in adults by around 14 days of age, the same time at which these neurons begin to display physiological and pharmacological responses similar to those seen in adults. Supported by MH-45286 and PHS RR 07059-24

431.12

WITHDRAWN

431.13

EFFECTS OF REPEATED SKF 38393 ON THE RESPONSIVENESS OF NIGROSTRIATAL SYSTEM NEURONS. M.D. Kelland, D.K. Pitts, A.S. Freeman and L.A. Chiodo. Lab. of Neurophysiology, Center for Cell Biology, Sinai Research Institute, and the Cellular and Clinical Neurobiology Program, Wayne State University School of Medicine, Detroit, Michigan 48235.

Extracellular single-unit and iontophoretic recording techniques were used to examine the effects of repeated SKF 38393 (15 mg/kg/day; 14 or 28 days) on nigrostriatal DA or Type I caudate neurons in the rat. Repeated SKF 38393 did not alter the basal activity of DA neurons or the potency of quinpirole to induce inhibition. However, 28-day SKF 38393 treatment eliminated the effects of acute SKF 38393 on both the rate-dependent nature of quinpirole-induced inhibition and sciatic nerve stimulation-induced inhibition of DA neurons. In contrast, one week after 28-day SKF 38393 treatment, quinpirole-induced inhibition by itself was no longer rate-dependent, an effect which was reversed by acute pretreatment with SCH 23390; these cells were also highly sensitive to acute D1 enhancement of the response to sciatic nerve stimulation.

28-day SKF 38393 treatment resulted in reduced sensitivity of Type I caudate neurons to iontophoretic SKF 38393, but enhanced sensitivity was observed one week later. Thus, chronic SKF 38393 can induce functional desensitization of D1 receptors, but one week withdrawal is followed by sensitization. (MH41557 [LAC], MH42136 [ASF], MH09781 [DKP], Sinai Res. Inst.; MDK is a Tourette Syndrome Association Postdoctoral Fellow)

431.14

EFFECTS OF CHOLECYSTOKININ (CCK) FRAGMENTS AND CR 1409 ON MIDBRAIN DOPAMINE NEURONS. A.S. Freeman, M.D. Kelland, J. Zhang* and L.A. Chiodo. Lab. of Neurophysiology, Center for Cell Biology, Sinai Research Institute, and the Cellular and Clinical Neurobiology Program, Wayne State University School of Medicine, Detroit, MI 48235.

Extracellular single-unit recording experiments were performed to further study the modulatory effects of CCK on midbrain DA cells. CCK-8S (8 ug/kg) preferentially excited non-bursting mesoaccumbens DA cells (7/7 cells), whereas only 1/12 bursting cells was excited. In support of this finding, CCK-8S induced burst-firing (with coincident excitation) in 7 out of 10 additional non-bursting cells. CCK-8S pretreatment (4 min) enhanced the potency of i.v. quinpirole to inhibit mesoaccumbens DA neurons (ED50= 2.7 $\pm$ 1.7 ug/kg vs. 5.7 $\pm$ 1.8 ug/kg in control rats). CCK-8US and CCK-4 were without effect.

The CCK-A receptor antagonist CR 1409 (.05 or .5 mg/kg, i.v.) did not alter the sensitivity of mesoaccumbens DA neurons to quinpirole (ED50 = 5.7 $\pm$ 1.7 and 5.6 $\pm$ 1.7 ug/kg respectively). In the cell per track paradigm, repeated administration of CR 1409 (14 days, .5 or 5 mg/kg/day, i.p.) increased the numbers of spontaneously active DA cells in the A10 region. These results suggest a modulatory role for CCK-A receptors on DA cell activity. Ongoing experiments continue to test this possibility. (Supported by MH42136 [ASF], MH41557 [LAC] and Sinai Res. Inst.; MDK is a Tourette Syndrome Association Fellow.)

431.15

THE EFFECTS OF THE PHENCYCLIDINE ANALOGUES BTCP AND TCP ON NIGROSTRIATAL DOPAMINE NEURONAL ACTIVITY. C. Rouillard, L.A. Chiodo and A.S. Freeman. Lab. of Neurophysiology, Center for Cell Biology, Sinai Research Institute and the Cell. & Clin. Neurobiology Prog., Wayne State Univ., Detroit, MI 48235.

Extracellular single-unit recording techniques were used to evaluate the effects of two PCP derivatives, BTCP and TCP, on the electrophysiological activity of identified nigrostriatal dopamine (NSDA) neurons in anesthetized rats. Intravenous BTCP produced a dose-dependent decrease in the firing rate of NSDA neurons whereas TCP induced an activation of cell firing at low doses followed by a reversal of the response with larger doses. A hemitransection of the brain anterior to the substantia nigra significantly reduced the inhibitory effect of BTCP while this procedure did not affect the response to TCP. However, iontophoretic application of BTCP induced a current-dependent inhibition of NSDA cells while local application of TCP had no effect on the firing rate of these cells. These data indicate that PCP analogues are able to interact with the nigrostriatal DAergic pathway through distinct and opposing mechanisms. The results will be discussed in light of recent observations that BTCP is selective for the DA uptake site while TCP is selective for the high-affinity PCP binding site. Supported by MH42136 (ASF), MH41557 (LAC) and Sinai Res. Inst.; CR is a FRSQ postdoctoral fellow.

431.16

THE EFFECTS OF SIGMA LIGANDS ON THE FIRING RATE OF IDENTIFIED DOPAMINERGIC NEURONS. J. Zhang*, L.A. Chiodo and A.S. Freeman. Lab. of Neurophysiology, Center for Cell Biology, Sinai Research Institute and the Cell & Clin. Neurobiology Prog., Wayne State Univ., Detroit, MI 48235.

Extracellular single-unit recording techniques were used to examine the effects of ligands for the high-affinity sigma binding site on the electrophysiological activity of identified nigrostriatal (NS) and mesoaccumbens (MA) dopamine (DA) neurons in anesthetized rats. (+)-pentazocine (1-16 mg/kg, i.v.) produced either no effect or weak excitations of NSDA and MADA cells while DTG was without effect at doses up to 2 mg/kg. Higher doses of DTG resulted in respiratory depression and death. The putative sigma antagonist, BMY 14802, dose-dependently (0.25-8 mg/kg) increased NSDA cell firing rate. Although (+)-3-PPP has higher affinity for the sigma site compared to DA receptors, this compound is known to exert many effects typical of a DA agonist. In contrast to the other sigma ligands, (+)-3-PPP (0.04-0.64 mg/kg) inhibited NSDA cell firing rate in a dose-dependent manner. Preliminary data suggest that (+)- but not (-)-butaclamol can reverse this effect of (+)-3-PPP which implies that the sigma binding properties of (+)-3-PPP are not responsible for the inhibition. Thus, a coherent explanation of the relationship between sigma sites and DA cell physiology remains elusive. (Supported by MH 42136, MH 41557 and Sinai Research Institute.)

431.17

ELECTROPHYSIOLOGICAL STUDIES OF NIGROSTRIATAL DOPAMINE NEURON ONTOGENY. D.K. Pitts, A.S. Freeman and L.A. Chiodo. Lab. of Neurophysiology, Center for Cell Biology, Sinai Research Institute, and the Cellular and Clinical Neurobiology Program, Wayne State University School of Medicine, Detroit, Michigan 48235.

The ontogeny of nigrostriatal dopamine (NSDA) neurons was examined in chloral hydrate-anesthetized 2-week(WK)-old (n=44) and adult (AD; 8-10 WKS old, n=40) rats using standard extracellular recording techniques. NSDA neurons from 2WK-olds were found to have significantly lower basal discharge rates and conduction velocities and fewer cells exhibited a bursting pattern (2WK: 11.4%; AD: 42.5%) compared to adults. 2WK-olds were less sensitive than adults to the inhibitory effects of IV apomorphine on discharge rate. Neither midbrain/forebrain hemitransections nor SCH 23390 pretreatment eliminated the apomorphine response differences indicating a lack of involvement of forebrain DA D1 receptors. No significant differences between IV quinpirole dose-response curves were observed at these two ages suggesting similar DA D2 somatodendritic autoreceptor sensitivity. Iontophoretic studies comparing NSDA neuron sensitivity to apomorphine and quinpirole are currently underway.

(Supported by MH41557 [LAC], MH42136 [ASF] and MH09781 [DKP] and Sinai Research Institute.)

431.18

RETROGRADELY LABELED DOPAMINERGIC NEURONS IN PRIMARY CULTURES FROM NEONATAL RATS. R. Shen, L. A. Chiodo and G. Kapatos. Labs. Neurophysiology and Neurochemistry, Ctr. Cell Bio., Sinai Res. Inst., and Cell. and Clin. Neurobiol. Prog., Wayne State Univ., Detroit, MI 48235.

We have developed a primary cell culture system for mesencephalic neurons from neonatal rats. Dopaminergic (DA) neurons in these cultures may be identified by fluorescent latex microspheres (FLMs) in the soma and dendritic processes. The fluorescent latex microspheres were bilaterally injected to the caudate nucleus in 1-day old rat pups. After two days, slices of mesencephalon were removed and the substantia nigra (SN) region was dissected out. The brain tissue was dissociated and numerous cells were found to contain FLMs. Cells were plated on an astrocyte feeder layer prepared from cortical tissues of 1-3-day old rat pups. Immunohistochemistry revealed that the neurons labeled with FLMs also showed positive tyrosine-hydroxylase (TH) histofluroscence both in the culture as well as in thin tissue sections containing the SN. We found that the majority of TH-positive neurons in the SN were labeled by the microspheres 28 hours after the injection. These neurons have either fusiform or multipolar cell bodies and long processes with varicosities.

Thus, this culture system allows for the study of the molecular biology of identified nigrostriatal DA neurons using a variety of techniques.
(MH41557 [LAC], NS26081 [GK],and Sinai Res. Inst.)

431.19

LINEAR MODE STRUCTURE OF SUBSTANTIA NIGRA (SN) DOPAMINERGIC (DA) NEURONAL ACTIVITY. J.H. Carlson, M.P. Paulus and S.L. Foote. UCSD Dept. Psychiatry (M-003), La Jolla, CA 92093.

We have described a significant tendency for interspike intervals (ISIs) of SN DA neurons to oscillate between short and long ISIs. To test the hypothesis that there is a cyclical, rate-independent structure in DA neuronal discharge patterns, a linear mode analysis was conducted using the Fast Fourier Transform (FFT) of the ISIs. The data were normalized with respect to the mean before calculating the FFT. The FFT for each data set of 500 ISIs was obtained multiple times by dividing the data into segments of 128 ISIs, repeatedly computing the FFT, and shifting the data segment using a lag of 64, to reduce the variance of the resulting peaks in the power spectrum. Data were shuffled 100 times and a power spectrum was obtained for each shuffled data set. A *significant cyclical component* (SCC) was defined as any cycle in the power spectrum of the original data set that exceeded the power of that cycle from the shuffled data $\geq$95 times. In most neurons, SCCs agreed with our previous description of short-long oscillations, i.e. the power spectrum revealed significant 2-3 cycles. However, additional SCCs indicated longer cycle lengths and greater variety in the structure of discharge patterns among DA neurons. After local infusion of nicotine, a reduction, a preservation or an increase in the mode structure were found, depending on the SCCs in the baseline condition. Thus, the variation in ISI sequences of SN DA neurons is not "random" but contains SCCs.

431.20

LOCALLY PERFUSED MORPHINE INHIBITS STRIATAL DA RELEASE: REVERSAL BY SYSTEMIC MORPHINE. Z.L. Rossetti, S. Carboni, F. Melis, N.H. Neff* and G.L. Gessa, Dept. of Neuroscience, University of Cagliari, Italy and *Dept. of Pharmacology, Ohio State University, Columbus OH, U.S.A.

The systemic administration of morphine to rats activates DA cell firing activity. The activation of DA neurons induces the release of the neurotransmitter in the terminal areas. Accordingly, by using the brain dialysis technique, we measured elevated (ca 200% of baseline) DA concentrations in perfusates from rat ventral striata following systemic morphine (10 mg/kg s.c.). However, when we perfused morphine into the caudate of the awake rat through the dialysis fibre, a concentration dependent (1-100 μM) inhibition of DA release (to 60-30% of baseline) was produced. This effect was antagonized by systemic naloxone (2 mg/kg i.p.). When striatal DA release was inhibited by *locally perfused* morphine, *systemic* administration of morphine (10 mg/kg s.c.) reverted the inhibitory effect, DA release being stimulated to about 200% of baseline. Conversely, when naloxone (0.1 μM) was perfused locally through the striatum, and morphine was administered systemically, DA release was markedly stimulated (over 400% of baseline). Naloxone did not affect *per se* DA release. Thus, systemic morphine and locally perfused morphine affect DA release in opposite directions and these effects are mediated by opiate receptors. These results suggest that morphine has antithetic effects on DA transmission: an inhibitory action in the terminal area, and a stimulatory one in the somato-dendritic region. It is possible that an unbalance between these two actions may explain the paradoxical effects of opiates on DA system observed in opiate tolerance and dependence.

CATECHOLAMINES V

432.1

DOSE DEPENDENT EFFECTS OF HALOPERIDOL ON STIMULATED DOPAMINE RELEASE IN THE STRIATUM *IN VIVO*. T.A. Patterson and J. O. Schenk. Program in Biochemistry and Dept. of Chemistry, Washington State University, Pullman, WA 99164-4630.

In vitro studies testing the effects of haloperidol (HAL) on stimulated DA release have resulted in different effects. HAL has been found to both increase and decrease the amount of stimulated dopamine (DA) released from rat striatum depending on the dose used. This led to an interest in whether HAL could have dose dependent effects on DA release *in vivo*.

In vivo voltametry at 30 um carbon fiber electrodes, was used to obtain a dose response curve for the effect of HAL on stimulated DA release. The electrode, with a syringe glued 1 mm away, was implanted into the striatum of a chloral hydrate anaesthetized rat. A control stimulus was elicited with a 20 nanomole injection of K^+ through the syringe. An ip injection of HAL was then given and the electrode assembly moved to the contralateral striatum. One hour later, a second K^+ stimulus was given. It was found that at low doses, ($\leq$ 0.05 mg/kg) there was no change in DA release, at medium doses (0.25 - 0.50 mg/kg) there was an increase in DA release, and at high doses (5.0 mg/kg) release was slightly decreased relative to control stimulus magnitudes. Correlations between these results, tissue [HAL] *in vivo*, behavioral measures and *in vitro* studies will be presented. (Support by NIMH Grant 42759 and the state of Washington).

432.2

AMPHETAMINE EXERTS ANOMALOUS EFFECTS ON DOPAMINERGIC NEURONS IN NEONATAL RATS F. Trent, S. Nakamura, and J.M. Tepper, Department of Biological Sciences and Center for Molecular and Behavioral Neuroscience, Rutgers University, Newark, NJ, 07102.

Amphetamine (AMP) and related stimulants increase arousal and locomotor activity in adults of many species. These drugs also suppress the spontaneous activity of nigrostriatal dopaminergic (NSDA) neurons. In hyperkinetic children however, stimulants exert a paradoxical behavioral effect, ameliorating hyperkinesis and increasing attention span. In this abstract, we describe the effects of AMP on the neuronal activity of NSDA neurons in neonatal rat pups ranging in age from the day of birth (PD1) through PD28.

Extracellular recordings from antidromically identified spontaneously active NSDA neurons were obtained in urethane-anesthetized (1.3 mg/g, i.p.) Sprague-Dawley rat pups. After obtaining a stable baseline, 5 mg/kg AMP was administered i.p. The effects of AMP were analyzed at 5 minutes post-injection. In PD1-6 rat pups, AMP produced a significant *increase* in spontaneous firing rate (mean $\pm$SEM = +183.6$\pm$84.9%, n=5, p< .001). In PD7-15 pups, there was no change in the mean firing rate (-8.2$\pm$12.5%, n=3, p> .05). In PD16-28 pups, AMP exerted a significant inhibitory effect (-39.8$\pm$5.9%, n=8, p< .001). In three additional cases in PD1-6 rats, apomorphine (1 mg/kg, i.p.) caused a near complete inhibition of spontaneous firing (-99.0$\pm$1.0%, p<.001). The timing of the development of the inhibitory response to AMP coincided with that of the most marked postnatal changes in the cytoarchitecture of substantia nigra, as well as that of a transient shift in the firing pattern of NSDA neurons to a pacemaker-like mode and a transient decrease in the duration of post-stimulus inhibition evoked from neostriatum.

These data demonstrate paradoxical effects of AMP on NSDA neurons in early postnatal rats that may be related to its paradoxical behavioral effects in human children. This effect does not appear to be due to decreased autoreceptor function, but may be due to an altered ability of AMP to release DA from nigral dendrites, and/or an altered response to AMP in telencephalic terminal fields. The normal inhibitory response to AMP begins to develop at around two weeks of age following a period of structural and physiological reorganization of substantia nigra. Supported by MH-45286 and PHS RR 07059-24.

432.3

ALTERATIONS IN STEREOTYPIC BEHAVIOR AND DOPAMINE RECEPTOR BINDING FOLLOWING CHRONIC ASCORBIC ACID-HALOPERIDOL TREATMENT. J.K. Rowlett[a], R.C. Pierce[b], M.T. Bardo[a], G.V. Rebec[b]. [a]Dept. Psychology, Univ. Kentucky, Lexington, KY 40506 and [b]Prog. Neural Science, Dept. Psychology, Indiana Univ., Bloomington, IN. 47405.

The present study assessed the effects of ascorbic acid (500 mg/kg, i.p. for 21 days), haloperidol (0.5 mg/kg, s.c. for 21 days) or ascorbic acid-haloperidol combination (500 mg/kg and 0.5 mg/kg, respectively, for 21 days) on apomorphine-elicited behaviors and D_2-dopamine receptor binding. Rats displayed significantly enhanced repetitive sniffing to apomorphine (0.5 mg/kg, s.c. 5 days after last chronic injection) after chronic ascorbic acid-alone and chronic haloperidol-alone treatments when compared to chronic vehicle. Chronic ascorbic acid-haloperidol treatment resulted in a repetitive sniffing response to apomorphine that was of greater magnitude than either chronic ascorbic acid-alone or haloperidol-alone treatment. As expected, [^{3}H]spiperone saturation studies on striatal tissue revealed an up-regulation of D_2-dopamine receptors in rats treated with haloperidol. However, no effect of ascorbic acid treatment was found on D_2 receptor density, suggesting that chronic ascorbic acid produces behavioral supersensitivity to apomorphine through mechanisms different from chronic haloperidol treatment.

Supported by NSF (BNS 87-11240).

432.4

ACUTE AND CHRONIC HALOPERIDOL INCREASE STIMULATED DOPAMINE RELEASE IN THE RAT CAUDATE NUCLEUS. D. J. Wiedemann, and R. M. Wightman. Dept. of Chemistry, Univ. of N. Carolina, Chapel Hill, NC 27599-3290.

In vivo voltammetry with Nafion-coated, carbon-fiber electrodes was used to measure dopamine (DA) overflow in the caudate nucleus of anesthetized rats. Overflow was elicited by electrical stimulation of the medial forebrain bundle with bipolar electrodes (300 μA, 120 pulses) with frequencies of 10 to 60 Hz. Acute haloperidol (0.5 mg kg^{-1}) caused DA overflow to increase at each frequency, with a maximum effect at 30 Hz of a two-fold increase. Following chronic haloperidol administration (0.5 mg kg^{-1} for 30 days), DA overflow was also increased relative to drug naive animals at all frequencies tested. The greatest increase, nearly 7-fold, occurred at 30 Hz. When the chronically treated animals were challenged with haloperidol, stimulated overflow was unchanged. In contrast, animals which were withdrawn from the drug for two weeks after chronic treatment were responsive to acute challenges of haloperidol in a manner similar to the acute animals. Thus, chronic haloperidol treatment leads to an increase in the amount of dopamine available for release, an effect that is reversible after drug withdrawal.

432.5

METHYLPHENIDATE AND PEMOLINE INHIBIT THE FIRING RATE OF RAT SUBSTANTIA NIGRA DOPAMINE NEURONS. A. Shenker*, D.A. Bergstrom and J.R. Walters. NIGMS, NINDS, NIH, Bethesda, MD 20892.

The stimulants *d*-amphetamine (AMP), methylphenidate hydrochloride (MPH) and pemoline (PEM) are the drugs most commonly used to treat Attention Deficit Hyperactivity Disorder (ADHD) in children. Efficacy of these drugs is thought to involve indirect agonism at brain dopamine (DA) receptors. Although the inhibitory effect of AMP on substantia nigra pars compacta DA neuron firing is well described (ED_{50} = 1 mg/kg), comparable studies of MPH and PEM have not been conducted.

Extracellular single unit recording of DA cell activity was performed in chloral hydrate anesthetized rats. MPH caused a dose-dependent inhibition of DA cell firing rate (ED_{50} = 1.3 ± 0.1 mg/kg i.v., n=10). Whereas AMP routinely produces 100% inhibition, maximal inhibition by MPH averaged 82 ± 10%, with only 3/10 cells totally inhibited. Inhibition by MPH was reversed by haloperidol (50 µg/kg i.v.). Pretreatment with reserpine (7.5 mg/kg i.p., 24 hrs), which depletes vesicular DA and blocks the behavioral effects of MPH, attenuated by 60% the inhibitory effect of 2.5 mg/kg MPH (n=4). PEM was much less potent than MPH. PEM (i.v., dissolved in 45% hydroxypropyl-β-cyclodextrin) caused no inhibition of firing at 5 mg/kg (n=8) and 36 ± 9% inhibition at 25 mg/kg (n=5). These results show that 3 drugs with therapeutic efficacy in ADHD share the ability to inhibit DA cell activity. Their relative potencies (AMP~MPH>PEM) parallel their known potencies as indirect DA agonists. The weak effect of PEM suggests that its clinical potency may not be due to dopaminergic actions alone.

432.6

THE D-1 AGONIST SKF 38393 SIGNIFICANTLY INHIBITS SUBSTANTIA NIGRA PARS COMPACTA DOPAMINE CELL ACTIVITY FOLLOWING RESERPINE TREATMENT. K.-X. Huang, D. A. Bergstrom, G.-Z. Jin* and J.R. Walters. NINDS, Bethesda, MD 20892 and Shanghai Inst. Materia Medica, Shanghai, China.

It is generally agreed that substantia nigra (SN) pars compacta dopamine (DA) neurons do not have D-1 receptors on their cell bodies. Thus, previous studies indicating that the D-1 antagonist SCH 23390 excites a subpopulation of these cells when administered to locally (but not systemically) anesthetized rats support the existence of a "long loop" feedback regulating DA cell activity and modulated by tonically stimulated postsynaptic D-1 receptors. To further explore the circuitry involved in this feedback and the functional expression of D-1 receptors, effects of the D-1 agonist, SKF 38393 were examined in rats treated for 6-8 days with 1 mg/kg reserpine, s.c. In control, locally anesthetized, artifically respired, gallamine-treated rats, 10 mg/kg i.v. SKF 38393 (n=5) exerted no significant effect on DA cell firing rate. However, in reserpine treated rats, the D-1 agonist dramatically inhibited DA cell activity by 69±12% (n=6). DA cell firing slowed gradually over the first 6 min after injection and remained suppressed for at least 30 min. This inhibition was reversed by SCH 23390, but not by haloperidol. Some effects were noted 3-7 hours after a single reserpine injection; 3 of 6 cells were markedly inhibited (ave. inhibition: 36 ± 19%, n=6). The time course and response of the DA cells to SKF 38393 in the 6-8 day reserpinized rats closely resemble the response of SN pars reticulata cells to SKF 38393 in 6-hydroxydopamine DA cell-lesioned rats (Weick and Walters, 1987) suggesting that both SN cell populations are similarly affected by pathway(s) influenced by supersensitive D-1 receptors.

432.7

RECOVERY OF DIURNAL LOCOMOTORY ACTIVITY AFTER MPTP CORRELATES WITH INCREASED CATECHOLAMINE SYNTHESIS BY SURVIVING NEURONS. N.A. Seniuk, C.E. Greenwood and W.G. Tatton. Depts. Physiology and Nutr.Sci., Univ. Toronto, Toronto, Ontario. M5S 1A8.

To determine how the loss of substantia nigra compacta (SNc) neurons and reductions in striatal dopamine (DA) (Seniuk et al., Br. Res., In Press,1990) relate to a quantitative measure of motor performance, the locomotory movements of 5 week old C57Bl mice were continuously monitored for 104 days following a 20 day entrainment period to a 12:12 hour light:dark (LD) cycle. Control locomotory activity was recorded for 14 days during LD and then dark:dark (DD) conditions. The mice were then injected with saline or MPTP (37.5,75,150,300 mg/kg) over a period of 5 days and their activity was followed for a further 90 days (d5-d95). Hourly activity counts for periods of120 hours were used for Fourier analysis. For the control activity, major power peaks extended from 21-25 hours/cycle (h/c- centered at 24 h/c for LD conditions and 23.85 h/c for DD conditions) with smaller, secondary peaks at 75 h/c and125 h/c. The area under the 21-25 h/c power peaks was unchanged relative to controls for d5-d10 for saline, 37.5 and 75 mg/kg MPTP animals while 150 and 300 mg/kg animals were reduced to 30-60% of control. The power for the 21-25 h/c peak gradually recovered to control over d10-d20 and remained at control levels to d95. Measurement of tyrosine hydroxylase (TH) immunodensity in the SNc neuronal somata and DOPAC/DA ratios in the striatum at corresponding days after saline or MPTP treatment showed that reduced TH immunodensity and DOPAC/DA ratios per average neuron correlated to the reduced power of the 21-25 h/c peaks over d5-d20. Recovery of the peak power to control levels corresponded to increased TH immunodensity in surviving SNc neurons (mean of 160% of control somata values) and increased DOPAC/DA ratios per average surviving neuron (mean of 360% of control). We propose that behavioral recovery following MPTP exposure is in part due to compensatory increases in catecholamine synthesis by surviving neurons.(Parkinson Foundation of Canada and MRC Canada MT5218.)

432.8

EVALUATION OF THE EFFECTS OF SIGMA BINDING LIGANDS ON NIGROSTRIATAL DOPAMINERGIC FUNCTION. L.T. Meltzer, C.L. Christoffersen, K.A. Serpa and T.G. Heffner, Department of Pharmacology, Parke-Davis Pharmaceutical Research Division, Warner-Lambert Co., Ann Arbor, MI 48105.

The identification of haloperidol (HPD) sensitive sigma binding sites and the suggestions of their involvement in antipsychotic drug action have stimulated interest in the effects of selective sigma ligands on brain dopamine (DA) systems. The present studies assessed the ability of sigma ligands to alter the firing activity of A9 DA neurons recorded extracellularly in anesthetized rats and to elicit extrapyramidal side effects (EPS) in monkeys that are sensitized to the acute dystonic effects of HPD. The selective sigma ligands DTG (2.0 mg/kg i.v.) and (+)-pentazocine (16 mg/kg i.v.) produced a 10% decrease and a 20% increase, respectively, in DA neuron firing. In contrast, BMY 14802 (6.4 mg/kg i.v.), a compound which has weaker affinity for sigma binding sites than DTG or (+)-pentazocine, increased DA neuron activity by 52%. This effect of BMY 14802 may be due to its greater affinity for 5-HT1A receptors than DTG and (+)-pentazocine. (+)-Pentazocine (5-10 mg/kg s.c.) did not induce EPS in HPD-sensitized monkeys. These studies do not reveal a consistent influence of sigma ligands on nigrostriatal DA function.

432.9

EFFECTS OF DOPAMINE AUTORECEPTOR AGONISTS ON DOPAMINE AND ITS METABOLITES AS MEASURED BY MICRODIALYSIS IN THE PRIMATE CAUDATE PUTAMEN. L.W. Cooke, F.W. Ninteman*, T.G. Heffner and M.D. Davis. Department of Pharmacology, Parke- Davis Pharmaceutical Research Division, Warner-Lambert Co., Ann Arbor, MI 48105.

Dopamine (DA) autoreceptor agonists are being explored as possible drugs for the treatment of schizophrenia. Although intracerebral microdialysis (ICMD) is employed increasingly for the measurement of DA and DA metabolites in rats, it has been used less frequently in primates. We have developed techniques for ICMD in the caudate putamen of squirrel monkeys. Bilateral chronically-implanted guide cannulae were used to permit repeated acute implants of dialysis probes into the putamen of awake chaired monkeys. Several DA autoreceptor agonists, including B-HT 920 and PD 128483, decreased the levels of DA in putamen dialysates. Certain of these neurochemical effects were comparable in magnitude and duration to behavioral effects observed in a conditioned avoidance test in the same species. Repeated ICMD measurements at a single site in the same animal over a period of months revealed no appreciable decrement in basal dialysate DA levels or in measured drug response. These studies indicate that DA autoreceptor agonists can produce inhibition of DA release from central neurons in primates and demonstrate that ICMD can be employed to quantify the actions of such drugs in primates in vivo.

432.10

STRIATAL INFUSION OF SEROTONIN (5HT), TFMPP, OR m-CPP FACILITATE DOPAMINE RELEASE IN-VIVO. S. Benloucif, M.J. Keegan*, and M.P. Galloway, Lafayette Clinic, Cellular and Clinical Neurobiology Program, Dept. of Psychiatry, Wayne State Univ. School of Medicine, Detroit, MI 48207.

We reported previously that striatal infusion of the 5HT-1 agonists TFMPP, RU24969, or 8-OHDPAT increased extracellular DA in-vivo when measured with microdialysis. Recent work has focused on the mechanism associated with the putative facilitation of DA release by 5HT. Agents were dissolved in artificial CSF and infused into the anterior lateral striatum of chloral hydrate anesthetized rats via the microdialysis probe. Drug-induced changes in levels of dopamine (DA) and metabolites from both striata were monitored by HPLC-EC. In addition to the facilitation afforded by TFMPP and RU24969, extracellular DA increased 3 - 10 fold with infusion of 5HT (10 µM, n = 6) and the major metabolite of trazadone, m-CPP (100 µM, n = 4). TFMPP (1 mM) did not alter the S2/S1 ratio of DA release from two K^+ pulses (100 mM in the perfusate) suggesting that facilitation occurs only with release induced by Na^+ dependent neuronal depolarization. Consistent with this interpretation, TTX (1 µM) decreased the effect of 5HT on DA release by 60% (n = 3). TFMPP's facilitation of DA release was not reduced by the 5HT reuptake inhibitor sertraline (10 µM), indicating that TFMPP does not act via the 5HT transporter (n = 4). Increasing extracellular DA with nomifensine decreased the effect of 5HT, suggesting that either DA autoreceptor stimulation by endogenous DA or inhibition of DA reuptake blocked the facilitation by 5HT. (Supported by MH-41227, DA-04120, and the State of Michigan Dept. of Mental Health).

432.11

PREVENTION OF 6-OHDA-INDUCED STRIATAL DOPAMINE (DA) RECEPTOR SUPERSENSITIVITY BY DAILY INJECTIONS OF A D1 RECEPTOR AGONIST X.-T. Hu and F.J. White. Dept. Psychiat., Wayne St. Univ. Sch. Med., Lafayette Clinic, Detroit, MI 48207

Behavioral and electrophysiological studies were conducted to determine the extent to which D1 receptor supersensitivity is involved in apomorphine (APO)-induced contralateral turning and the supersensitive responses of caudate-putamen (CPu) neurons in rats with unilateral 6-OHDA lesions of the nigrostriatal DA system. APO (2mg/kg, sc) induced strong contralateral turning 7 days after injection of 6-OHDA (not vehicle) into the CPu. However, the turning responses to APO were almost completely abolished by a daily repeated treatment with the selective D1 agonist SKF-38393 (SKF)(8mg/kg, s.c. for 6 days). Single-unit recordings indicated that, although the inhibitory responses of CPu neurons to iontophoretic SKF and the selective D2 agonist quinpirole were both significantly enhanced in 6-OHDA (not sham)-lesioned rats, daily treatment with SKF effectively prevented the supersensitive responses of CPu neurons to both D1 and D2 agonists. In addition, while 6-OHDA lesions typically abolish the synergistic interaction between D1 and D2 receptors, such interactions were still present in 6-OHDA rats that received daily SKF injections. These findings indicate that the D1 receptor plays a critical role in the development of denervation supersensitivity and in the coupling of D1 and D2 receptor-mediated responses (Supported by APDA, USPHS Grants DA-04093 and MH-40832 to FJW).

432.12

FACTORS CONTROLLING THE STIMULATED OVERFLOW OF STRIATAL DOPAMINE MEASURED BY IN VIVO MICRODIALYSIS. D.H. Schwartz, I. Creese, and J. M. Tepper, Center for Molecular and Behavioral Neuroscience, Rutgers University, Newark, NJ 07102.

Impulse dependent dopamine (DA) release from nerve terminals is considered to be frequency and calcium dependent. Techniques to directly monitor basal and physiologically relevant stimulation-induced extracellular DA *in vivo* have only recently become available. The effects of medial forebrain bundle (MFB) stimulation on striatal DA overflow was examined using *in vivo* microdialysis in urethane anesthetized (1.3 g/kg) rats. A concentric microdialysis probe (0.25 x 4 mm) was inserted into the lateral portion of the neostriatum and a bipolar stimulating electrode was lowered into the MFB. In addition, single unit recordings were obtained from nigrostriatal DAergic neurons. A stimulating electrode coupled to the dialysis probe was used to antidromically identify DA neurons. Cells were then tested for antidromicity from the MFB to demonstrate that MFB stimulation was effectively eliciting action potentials in DA axons. Following the recording session, trains of 5 or 10 pulses (250 -500 µsec; 0.5 - 4 mA) were delivered to the MFB every 1.5 sec at varying frequencies (3.33 - 100 Hz) for 12 minute dialysis samples preceded by 1 or 2 12 minute non-stimulated baseline samples.

When the perfusate contained 1.2 mM calcium, even intense, high frequency stimulation (i.e. 10 pulses at 4 mA; 25-100 Hz) produced only small and non-significant elevations in extracellular DA despite the fact that amphetamine (0.5 mg/kg i.v.) and cocaine (5 mg/kg i. v.) produced dramatic increases. Addition of nomifensine to the perfusate did not significantly enhance stimulation-induced increases at this calcium concentration. In contrast, when the perfusate contained 2.4 mM calcium, there were significantly larger stimulation-induced increases in DA overflow in the absence of uptake blockade even at modest stimulus intensities and frequencies (5 or 10 pulses at 0.5 -1.5 mA; 3.33 - 12.5 Hz) ($F(1,13) = 26.95$; $p < 0.0003$). There was a significant correlation between the proportion of DAergic neurons that could be antidromically driven from the MFB and the increase in DA overflow induced by MFB stimulation across animals ($r = .823$; $df = 7$; $p < .007$). Surprisingly, MFB-stimulated DA overflow was not frequency dependent under these conditions. These data provide good evidence that *in vivo* microdialysis is sensitive to stimulation-induced increases in striatal DA overflow and that calcium concentration is a critical factor in its detection. Supported by MH-45286 and a Rutgers University Council Grant (JMT) and MH-32990 and DA-04612 (IC).

432.13

EFFECT OF 3,4-METHYLENEDIOXYMETHAMPHETAMINE (MDMA) ON CATECHOLAMINES IN FEMALE RAT CAUDATE AS MEASURED IN EXTRACELLULAR MICRODIALYSATE AND TISSUE HOMOGENATE. B. Gough, R.R. Holson, S.F. Ali and W. Slikker, Jr. Division of Reproductive and Developmental Toxicology, National Center for Toxicological Research, Jefferson, AR 72079.

Extracellular levels of dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), 5-hydroxyindoleacetic acid (5HIAA), serotonin (5HT) and homovanillic acid (HVA) were assayed in the caudate of freely moving rats using microdialysis and high performance liquid chromatography with electrochemical detection (HPLC-EC). Dialysates were assayed at 20 minute intervals for 4 hours after an I.P. injection of MDMA (10 mg/kg). In a separate study, animals were injected I.P. with MDMA (10 mg/kg) and at 20, 60, 120 and 180 minutes after treatment, animals were sacrificed, whole brains removed and caudate homogenates prepared. MDMA elicited an amphetamine-like increase in DA release, followed by an increase in DA synthesis. DOPAC and HVA were both reduced in dialysate, while only DOPAC was reduced in homogenate. 5HT release was also increased, followed by a drop in caudate homogenate levels by 3 hours. It is concluded that the acute effect of MDMA on caudate is greater on the DA than on the 5HT system. DA extracellular content was 686% of control at 80 minutes; homogenate release was 122% at 120 minutes. 5HT extracellular release was 123% at 20 minutes, then decreased thereafter.

432.14

HETEROGENEITY OF MESOACCUMBENS DOPAMINE NEURONS. Stephen Rayport, David Sulzer and Wei-Xing Shi. Depts. Psychiatry, Anatomy & Cell Biology, and Ctr. Neurobiology & Behavior, Columbia Univ.; Dept. Neuropathology, Psychiatric Inst., New York City 10032.

Midbrain dopamine (DA) neurons are increasingly recognized to be heterogeneous. We examined the intrinsic properties of single, identified, post-natal mesolimbic DA cells, isolated from synaptic influences, in low density cultures. Cells were taken from the ventral tegmental area of week-old rats, a stage by which the midbrain DA system shows a fair degree of maturity. A subset of mesolimbic neurons were identified (Rayport *et al.*, 1988) by retrograde labelling from the ventrolateral n. accumbens; 86% were dopaminergic. Mesoaccumbens cells showed four distinctive shapes: in decreasing order, cells were elliptical with an eccentric nucleus, fusiform, pyramidal or spherical. 55% contained the cotransmitter cholecystokinin, while 0% showed neurotensin staining (*cf.* Studler *et al.*, 1988). Mean resting potential was -55 mV, R-in was 630 megΩ and τ was 26 msec. Cells did not fire spontaneously (at 32° C) and had no recurrent connections, but often showed rhythmic membrane oscillations with depolarization leading to pacemaker firing. Full-size spikes, often triggered by low threshold Ca^{2+} spikes, had overshoots of 43 mV and widths of 1.2 msec (peak to *1/e*), overlapping considerably with unlabelled cells, which were about 25% dopaminergic. 89% of cells showed hyperpolarizing afterpotentials (mean = -4.5 mV), 64% showed time-dependent anomalous rectification, 84% showed low-threshold Ca^{2+} spikes, and 74% fired only after a latency of 100-200 msec. 59% showed spike-accommodation to long depolarizing steps. Cells exhibited a variable response to DA-agonist application. While the concatenation of several electrophysiological characteristics in individual labelled cells may identify them as dopaminergic (compare 86% dopaminergic with per cent of labelled cells showing a given characteristic), even a highly selected group of midbrain DA neurons exhibits considerable variation.

432.15

PREFRONTAL CORTICAL MODULATION OF MIDBRAIN DOPAMINE CELL ACTIVITY. *J. Grenhoff +, L. Pawlowski +, M. Herrera-Marschitz + and T.H. Svensson. Department of Pharmacology, Karolinska Institute, Stockholm, Sweden.*

The prefrontal cortex and the mesolimbobortical dopamine (DA) system originating in the ventral tegmental area (VTA) in the midbrain appear to play important roles in goal-directed behavior. Anatomical studies have demonstrated connections between these areas in several species.

In order to evaluate the functional role of these connections, effects of lesions of the medial prefrontal cortex (MPC) on the electrical activity of VTA-DA neurons were studied in the chloral hydrate-anesthetized male albino rat. Rats received kainic acid (KA) injections bilaterally in the MPC. After 7 days' recovery VTA-DA cell activity was recorded extracellularly and compared to sham-lesioned and untreated rats. VTA-DA cell firing rate was significantly increased in the KA group and the firing was more regular.

Previously, we have observed regularization of VTA-DA cell firing induced by reversible cold inactivation of the prefrontal cortex. The present findings further support the notion of a functionally significant influence from the MPC on VTA-DA neurons.

432.16

THE INVOLVEMENT OF D1 AND D2 RECEPTOR SUBTYPES IN THE EFFECTS OF NICOTINE ON DOPAMINE SYNTHESIS IN THE NUCLEUS ACCUMBENS AND STRIATUM. I.L. Holt and T.C. Westfall. Dept. of Pharmacol. St. Louis Univ. Med Ctr. St. Louis, MO 63104.

We have previously observed that nicotine (NIC, 1mg/kg s.c.) increases dopamine (DA) synthesis and tissue levels in the rat nucleus accumbens (NAc) and decreases DA synthesis and tissue levels in the striatum. In the present studies we have examined the involvement of D1 and D2 receptor subtypes in the NIC effects using the D1 antagonist SKF-83566 and D2 antagonist sulperide (SUL). DA synthesis was evaluated by measuring DOPA accumulation following inhibition of DOPA decarboxylase with NSD-1015 (100 mg/kg i.p. for 30 min). Tissues were homogenized and DOPA and DA levels were assayed by HPLC-EC. In the NAc, SUL (30 mg/kg i.p.) alone increased DOPA accumulation and prevented the NIC induced increase in DOPA. SUL decreased DA tissue levels and NIC effects were also attenuated. SKF alone had no effects on DOPA and attenuated the NIC induced increase in synthesis; DA levels decreased with SKF and decreased further with SKF and NIC. SUL in the striatum increased DOPA, while NIC continued to suppress synthesis in the presence of SUL. Striatal DA decreased with SUL and further with NIC. SKF decreased DOPA and DA in the striatum and attenuated NIC effects; NIC did not decrease levels of DOPA or DA further after SKF administration. The effects of NIC on DA synthesis in the NAC appear to involve both D1 and D2 receptors, while the striatal NIC effects appear to involve only D1 activity. (Supported by DA 02668.)

432.17

ELECTROPHYSIOLOGICAL EFFECTS OF REPEATED EXPOSURE TO SELECTIVE D1 AND D2 DOPAMINE AGONISTS ON THE MESOACCUMBENS DOPAMINE SYSTEM. D.J. Henry and F.J. White. Neuropsychopharm. Lab, Dept. Psychiatry, CCN Program, Wayne St. Univ. Sch. Med., Lafayette Clinic, Detroit, MI 48207.

Behavioral studies have indicated that repeated administration of non-selective dopamine (DA) agonists can result in sensitization to DA-mediated behavior. We have recently demonstrated enhanced D1-mediated grooming behavior following repeated administration of the selective D1 agonist SKF 38393 (SKF). The present electrophysolgical study sought to determine whether repeated administration of D1 and D2 selective agonists alters the sensitivity of the mesoaccumbens DA system. Male rats were given twice daily injections of the selective D1 agonist SKF 38393 (8 mg/kg, i.p.) or the selective D2 agonist quinpirole (QUIN, 0.5 mg/kg, i.p.) for 14 days. Control rats received equivalent injections of vehicle (DI water) twice daily. All rats were tested 16-24 hrs after the final injection. Extracellular single-unit recording techniques were used to determine the sensitivity of A10 DA neurons to "autoreceptor-selective" doses of i.v. apomorphine (APO). Subsensitivity to the inhibitory effects of APO was observed in QUIN pretreated animals. Preliminary results suggest a slight increase in sensitivity to i.v. APO in SKF treated rats. Extracellular recording techniques were used in combination with iontophoresis to examine responses of nucleus accumbens (NAc) neurons from pretreated animals to directly applied SKF and QUIN. Significantly enhanced inhibitory responses of NAc neurons to SKF (but not QUIN) were observed in animals receiving repeated SKF injections. Preliminary results indicate decreased responses of NAc neurons to iontophoretic QUIN (but not SKF) in animals receiving repeated QUIN injections. Supported by MH 40832 and DA 04093 to FJW and the State of Michigan.

432.18

EFFECTS OF REPEATED MORPHINE TREATMENT ON THE SENSITIVITY OF DOPAMINE AUTORECEPTORS: ELECTROPHYSIOLOGICAL STUDIES. Michael Jeziorski and Francis J. White, Neuropsychopharmacology Lab, Lafayette Clinic, Cellular and Clinical Neurobiology Program, Dept. of Psychiatry, Wayne State University School of Medicine, Detroit MI 48207.

When acutely administered at low doses, morphine and other opioid compounds elicit increases in locomotion in rats. Repeated administration of morphine produces an enhanced behavioral response; current evidence indicates that this behavioral sensitization may involve changes in the functioning of the mesoaccumbens (A10) dopamine (DA) system. Because impulse flow in A10 DA cells is regulated by somatodendritic DA autoreceptors, the following experiments were designed to investigate whether alterations in A10 DA autoreceptor sensitivity are associated with the modified responses to repeated morphine administration. The sensitivity of impulse-regulating somatodendritic DA autoreceptors was measured in chloral hydrate-anesthetized rats using single unit electrophysiological recording techniques. In rats pretreated with morphine on a schedule reported to induce behavioral sensitization (10 mg/kg/day for 14 days), the ability of systemic administration of the D2 DA receptor agonist quinpirole to inhibit DA cell firing via autoreceptor stimulation was not significantly different from the inhibition produced by quinpirole in control rats. A similar result was obtained with iontophoretic experiments, as locally applied DA produced a comparable degree of inhibition of A10 DA cell firing in control and morphine-treated rats. It appears, therefore, that sensitized responses to morphine are not associated with alterations in the sensitivity of somatodendritic DA autoreceptors on A10 DA cells. Subsequent experiments will examine the potential modulation of postsynaptic DA receptor sensitivity in response to repeated morphine. (Supported by USPHS grants DA-04093 and MH-40832 and by the State of Michigan.)

432.19

CONTINUOUS AND INTERMITTENT COCAINE TREATMENTS DECREASE SENSITIVITY OF THE A10 SOMATODENDRITIC DOPAMINE AUTORECEPTOR. J.M. Ackerman and F.J. White. Neuropsychopharmacol. Lab., Dept. Psychiat., CCN Program, Wayne St. Univ. Sch. Med., Lafayette Clinic, Detroit, MI 48207.

Repeated cocaine administration in animals results in behavioral sensitization to locomotor stimulation and stereotypy caused by cocaine. However, it is unclear whether an intermittent or continuous treatment regimen of cocaine administration will produce behavioral sensitization or tolerance, a factor that may be relevant to human cocaine abuse. The mesoaccumbens dopamine pathway projecting from dopamine neurons in the ventral tegmental area (A10) to the nucleus accumbens is involved in behavioral sensitization and cocaine reward. We have previously shown that repeated cocaine administration in rats by twice daily 10 mg/kg i.p. injection for fourteen days caused a subsensitivity of impulse-regulating somatodendritic autoreceptors on A10 dopamine neurons. The present study investigated whether subsensitivity of the somatodendritic autoreceptor would also occur following administration of an equivalent amount of cocaine over the same time period delivered continuously. Male rats received cocaine (20 mg/kg/day s.c.) continuously for fourteen days via osmotic minipumps. Within twenty-four hours of pump removal, single-unit recordings of A10 dopamine neurons were obtained *in vivo* under chloral hydrate anesthesia using standard extracellular recording techniques. The ability of the dopamine agonist apomorphine to inhibit basal firing of these dopamine cells was decreased, indicating the existence of autoreceptor subsensitivity. The concentration of apomorphine required to inhibit basal firing by 50% (ID_{50}) was increased to the same degree as in rats treated intermittently with i.p. injections. In rats tested after seven days of pump removal, the ability of apomorphine to inhibit cell firing had returned to control levels. The identical result was obtained following daily i.p. injections. The results suggest that continuous exposure to a low dose of cocaine can produce a decrease in autoreceptor sensitivity that is equal to that obtained with intermittent injections of a higher dose.

432.20

CONTRIBUTION OF DOPAMINERGIC INPUTS TO DOPAMINE AGONIST-INDUCED RESPONSES OF VENTRAL PALLIDAL NEURONS. R.J. Maslowski, D. An* and T.C. Napier, Dept. Pharmacol., Loyola Univ. Chicago, Stritch Sch. Med., Maywood, Il 60153.

Effects of acute and chronic removal of dopaminergic neurons on D1- and D2-mediated rate changes of ventral pallidal (VP) neurons in chloral hydrate anesthetized rats were examined. Activity increased in 68% of VP neurons tested with i.v. SKF38393 (SKF, D1 dopamine agonist), with E_{max} = 160% of baseline rates and ED_{50} = 1.1mg/kg[1]. In contrast, the D2 agonist, quinpirole (QUIN)-induced dose-dependent decreases in 58% of neurons tested (E_{max} = 60%, ED_{50} = 7.6ug/kg).

In the present studies, 150mg/kg i.v. of *gamma*-butyrolatone (GBL) was used to reversibly block dopaminergic neuronal transmission and cumulative doses of SKF (0.1-25.6mg/kg) were injected. Six of 21 neurons tested ceased firing after GBL administration and were omitted from further analysis. Of the remaining 15 neurons, 47% increased firing (E_{max} = 282%, ED_{50} = 2.2mg/kg). There was a twofold increase in E_{max} for the SKF-induced response in GBL-pretreated *versus* untreated rats. Studies evaluating the contributions of dopaminergic neurons for D2-induced changes in VP neuronal activity are in progress.

Dopamine agonist-mediated responses of VP neurons recorded 7 days after 6-hydroxydopamine-induced lesions of the medial forebrain bundle were similar in response distribution and magnitude for SKF and QUIN (E_{max} = 196%, ED_{50} = 0.8mg/kg; and E_{max} = 51%, ED_{50} = 5.4ug/kg, respectively) to those observed for the agonists alone. Thus, acute blockade of dopamine transmission increases the magnitude of the predominant response to SKF, but chronic blockade allows for recovery to the untreated level of responsiveness. (Supported by MH45180.)

1. Maslowski and Napier, *Neurosci. Abstr.* 15:1014, 1989.

432.21

CONTRIBUTION OF THE NUCLEUS ACCUMBENS TO VENTRAL PALLIDAL RESPONSES TO SYSTEMICALLY ADMINISTERED D1 AND D2 DOPAMINE AGONISTS. T.C. Napier. Dept. Pharmacol. Loyola Univ. Chicago, Stritch Sch. of Med., Maywood, Il. 60153.

Intra-nucleus accumbens (NA) injections of dopamine (DA), or SKF38393 (SKF, D1 agonist) followed by quinpirole (QUIN, D2 agonist), (but not either drug alone) is sufficient to increase ventral pallidal (VP) neuronal firing[1]. VP neurons are sensitive to i.v. administered SKF or QUIN[2] as well as microiontophoretic applications of dopamine[3]. These responses may be due to an activation of DA receptors located within the NA (i.v. drugs) or on terminals of projections from the NA (i.v. and iontophoretic drugs). The present experiment was designed to determine if the NA is necessary for dopaminergic influences on VP neurons.

Single VP neurons recorded in vivo from male Sprague Dawley rats anesthetized with chloral hydrate were tested for sensitivity to i.v. administration of SKF (3.2mg/kg) or QUIN (100ug/kg), and to these drugs following microinjections of procaine into the NA (40ug/2ul). In agreement with our previous work, SKF increased activity in 69% of 26 neurons tested, and QUIN suppressed firing in 57% of 28 neurons tested. Thirty- nine of 50 VP neurons tested demonstrated rate changes following intra-NA procaine injections. Of those neurons that responded to procaine (n=32), 60% increased firing after SKF injections. In contrast, rate suppression typical of QUIN treatments was observed only in 31% of 18 neurons sensitive to intra-NA procaine.

These data suggest that D1 receptor activation resulting in rate increases of VP neurons is independent of the NA. However, at least a portion of the D2 receptors involved with VP suppression may be located on NA neuronal elements. (Work supported by MH45180).

1. Yang and Mogenson, Brain Res. 489:237, 1989.
2. Maslowski and Napier, Neurosci. Abstr. 15:1014, 1989.
3. Napier and Potter, Neuropharmacology 7:757, 1989.

432.22

BASAL EXTRACELLULAR DOPAMINE IN THE NUCLEUS ACCUMBENS OF THE RAT AND THE EFFECT OF PERFUSATE CALCIUM AS STUDIED BY IN VIVO MICRODIALYSIS. **L.H. Parsons, H.O. Pettit, J.B. Justice, Jr. Department of Chemistry, Emory University, Atlanta, GA 30322.**

In vivo studies have produced a wide range of estimated basal concentrations of dopamine (DA) in several brain regions. This study was designed to provide a more rigorous estimate of the extracellular concentration of DA in the N ACC. Stable levels of DA were measured using four different perfusate Ca^{+2} concentrations (0.0, 1.2, 2.4 and 3.6 mM Ca^{+2}) at four different perfusion flow rates (0.2, 0.6, 1.2 and 2.3 ul/min). Each animal was perfused at a given flow rate (n=3 for each group) with DA measurements obtained for each Ca^{+2} concentration. A two way analysis of variance indicated a significant difference between flow rates ($P<0.004$) and Ca^{+2} concentrations ($P<0.0001$) and that the degree of calcium-induced variation was dependent on flow rate ($P<0.01$). Following the method of Jacobson et al. (J. Neurosci. Meth. 15:263-268; 1985) these data were subjected to a non-linear least squares regression as a function of flow rate at each Ca^{+2} conentration. The intercepts of the curves (zero flow) yielded basal extracellular DA as a function of varying Ca^{+2} concentration independent of the dialysis probe sampling characteristics. A linear regression of these values provides a curve of basal DA as a function of dialysate Ca^{+2} concentration. At an extracellular Ca^{+2} concentration of 1.2 mM (Moghaddum and Bunney, J. Neurochem. 53:652-654; 1989) basal DA was found to be 4.8 nM in the N ACC. This curve also indicated that removing calcium from the perfusion medium resulted in a 81% decrease in basal perfusate DA levels, indicating that the DA sampled was of vesicular origin.

432.23

DIFFERENTIAL ACTIVATION OF POPULATIONS OF MESOAMYGDALOID DOPAMINE NEURONS BY CONDITIONED STRESS: ATTENUATION BY DIAZEPAM. M. Coco, J. Kilts* C. Kuhn, C. Kilts. Dept. of Psychiatry, Duke Univ. Med. Ctr., Durham, N.C. 27710

Populations of dopamine (DA) neurons in the rat brain may be selectively activated by stress. Attenuation of stress-induced activation of DA systems has been observed with acute diazepam (DZ) treatment, but has not been examined regarding conditioned stress. Also, a subset of mesoamygdaloid DA projections might be especially sensitive to the effects of mild stress such as conditioned stress and to attenuation by DZ.

We mapped the effect of conditioned stress on the concentration of homovanillic acid (HVA) in distinct amygdaloid nuclei and other areas and the effect of DZ (1 or 3 mg/kg) on the stress-induced response in drug-experienced subjects. The conditioned stress paradigm resulted in significant elevations in classical indices of stress, including serum corticosterone, prolactin and plasma epinephrine, which were attenuated by DZ. Conditioned stress-induced increases in the estimated activity of DA neurons were specific for particular mesoamygdaloid DA neurons. Conditioned stress-induced changes in the HVA concentration of amygdaloid nuclei were differentially antagonized by DZ. This suggests a preferential role for a subset of mesoamygdaloid DA projections in the response to psychological stress or in the coping response to stress. (Supported by NIMH MH-39967)

432.24

MODULATION OF MESOLIMBIC DOPAMINE OVERFLOW BY NEUROTENSIN FRAGMENTS APPLIED TO THE VTA OR NUCLEUS ACCUMBENS IN VIVO AND TO MESOACCUMBENS EXPLANTS IN VITRO. **M.D. Davis, L.W. Cooke and T.G. Heffner. Department of Pharmacology, Parke- Davis Pharmaceutical Research Division Warner-Lambert Co., Ann Arbor, MI 48105.**

Neurotensin (NT) is an endogenous tridecapeptide that has a broad range of physiological and behavioral actions. NT and its binding sites are associated with dopamine (DA) neuronal systems in brain and NT can produce certain antipsychotic-like effects. Intracerebral microdialysis was used to examine DA overflow from nucleus accumbens nerve terminals during the infusion of NT fragments into the VTA or nucleus accumbens (NA) of anesthetized rats through a second dialysis probe. VTA infusion of NT 1-13 and NT 8-13, but not NT 1-10 (0.1-10 uM) evoked increases of 25-120% in DA overflow measured in the NA. Similar effects on NA DA efflux were seen when these NT fragments were applied in the same concentrations to the cell body region of mesoaccumbens explant plugs maintained in vitro. NT 1-13 and 8-13 produced similar effects on NA DA overflow when infused directly into the NA but failed to increase DA efflux from mesoaccumbens explants when applied directly to NA nerve terminals in vitro. These results suggest that NT receptors in mesolimbic DA cell body regions can modulate DA release from NA nerve terminals both in vivo and in vitro but that direct effects of NT on DA terminals in the NA is less clear.

BEHAVIORAL PHARMACOLOGY: DOPAMINE AND HORMONES

433.1

HIGH-PROTEIN DIET MODULATES DOPAMINE-AND NON-DOPAMINE MEDIATED BEHAVIORS IN RATS. E.S. Onaivi, J.W. Brock, A. Hamdi, and C. Prasad. Neuroscience Lab, Pennington Biomed. Res. C., Baton Rouge, and Dept. of Med., LSUMC, New Orleans, LA 70808.

Consumption of foods with altered macronutrients is alleged to have profound effects on behavior in man. Using rats as model we have studied the effect of dietary protein on a number of behaviors. Three grps of rats were subjected to equicaloric diet: normal-(20%), low-(8%) and high- (50%) casein for 20 wks. The high-protein group was more responsive compared to normal and low-protein groups in sensorimotor function (8±1,16±3, 17± 2s latency respectively), negative geotaxis (6±2, 9±2, 9±1s latency respectively), locomotor activity (1599±105, 1390±121, 575±29 photocell counts/30 min., respectively), and nociception (7.4±0.9, 6.9±0.2, 4.5±0.5 seconds, respectively) tests. There was also reduced aversion in the elevated-plus maze test (39% increase in dwell-time and 89% increase in number of entries, $p<0.05$). Results suggest that high-protein diet may modulate dopamine and non-dopamine mediated behaviors. (Dept. of Army, grant # 1788G8023)

433.2

DIFFERENT RAPID JAW MOVEMENT (RJM) RESPONSE TO A D1 AGONIST IN RATS EXHIBITING AN INBORN DIFFERENCE IN SPONTANEOUS RJM. **H. Rosengarten*, J.W. Schweitzer*, R.J. Shoerman* and A.J. Friedhoff. Millhauser Laboratories, Dept. of Psychiatry, NYU School of Med., New York, NY 10016**

There is considerable variability in rats in the rate of spontaneous RJM and in sensitivity to the D1 agonist, SKF 38393. Sprague Dawley rats were selected on the basis of their level of RJM response to SKF 38393 (either below or above the median of their group), and classified as low or high RJM. These rats were inbred (high with high and low with low) through seven generations and the rate of spontaneous and SKF 38393 induced RJM was assessed as well as D1, D2 and S2 receptor density and dopamine (DA) and DA metabolite levels. The inbred high RJM group had significantly higher spontaneous and SKF 39393 induced RJM than the inbred low group. There were no differences in the biochemical measures except that the density of S2 receptors in the prefrontal cortex was higher in the low RJM group.

433.3

D-1 AGONIST (SKF 38393), BUT NOT A D-2 AGONIST, EXERTS A CHOLINERGICALLY-MEDIATED ANALEPTIC EFFECT IN PENTOBARBITAL-NARCOTIZED RABBITS. A. Horita and M.A. Carino, Univ. of Wash. Sch. of Med., Seattle, WA 98195.

In previous studies we demonstrated that a number of drugs and peptides produced analeptic activity in rats and rabbits by activating hippocampal and/or cortical cholinergic pathways. Among these was cocaine, a drug known to exert many of its effects via dopamine mechanisms. In order to determine whether dopamine might be involved in its analeptic effect, we investigated whether D-1 and/or D-2 agonists might produce a similar response. We found that the D-1 agonist, SKF 38393 (2-5 mg/kg iv) significantly shortened the duration of pentobarbital narcosis in rabbits. The D-2 agonist quinpirole (up to 2 mg/kg iv) was without effect. The analeptic effect of SKF 38393 (5 mg/kg) was blocked by atropine but not by methylatropine. The effect was also blocked by SCH 23390 (0.1 mg/kg) or raclopride (2 mg/kg), suggesting the need of both D-1 and D-2 receptor systems. These results indicate that D-1 receptor stimulation activates central cholinergic pathways involved in cholinergic arousal (analeptic) mechanisms, but also that an intact D-2 system is necessary for expression of the response. They also support our earlier finding that the cocaine-induced analeptic effect in pentobarbital-narcotized rabbits was mediated via a D-1 dopamine mechanism (supported by NIDA grant DA 4907).

433.4

D-1 BUT NOT D-2 DOPAMINE RECEPTOR STIMULATION ATTENUATES BEHAVIORAL SUPERSENSITIVITY INDUCED BY HALOPERIDOL. C. Marin and T.N. Chase. ETB, NINDS, NIH , Bethesda, MD 20892.

Chronic neuroleptic administration induces supersensitive behavioral response to dopamine (DA) agonists. This effect might reflect DA receptor proliferation due to long term pharmacologic blockade. Since chronic DA agonist exposure induces DA receptor down-regulation, we have examined whether selective D-1 or selective D-2 stimulation can reverse the neuroleptic-induced increase in apomorphine-induced stereotypy. Rats were treated with haloperidol (1 mg/kg, s.c.) for 21 days followed by 5 days of either the selective D-1 agonist SKF 38393 (10 mg/kg, i.p.), the selective D-2 agonist quinpirole (1 mg/kg, i.p.), a combination of both drugs at the same daily doses, or normal saline. Apomorphine-induced stereotypy (0.3 mg/kg, s.c.) was measured four days after cessation of the therapeutic regimens. SKF 38393 but not quinpirole attenuated stereotypies induced by haloperidol. This effect was potentiated with the simultaneous administration of both drugs. The results suggest that D-1 DA stimulation might have an important role in the attenuation of extrapyramidal side effects produced by chronic neuroleptic administration.

433.5

DOPAMINERGIC MODULATION OF PREDATORY ATTACK BEHAVIOR IN THE CAT. M.B. Shaikh, C.L. Lu, and A. Siegel. Dept. of Neurosciences, UMDNJ - New Jersey Medical School, Newark, New Jersey 07103.

Dopamine (DA) containing fibers are known to project to those regions of the limbic forebrain and brainstem which play a key role in the expression of quiet biting 'predatory' attack behavior (QBA) in the cat such as the lateral hypothalamus (LH) and periaqueductal gray. The present study provides evidence for the involvement of DA in the regulation of QBA. In five cats, QBA was elicited by electrical stimulation of LH utilizing monopolar electrodes. After stable baseline response latency values were established, the nonselective DA agonist, apomorphine [APO] (1.0, 1.4 and 1.8 mg/kg), was administered systemically and its effects upon QBA were identified. APO significantly facilitated QBA in a dose- and time-dependent manner. At the maximum dose level (1.8 mg/kg) APO infusion resulted in a 30% decrease in response latencies which lasted for 60 min postinjection. Systemic injections of the D2 receptor antagonist, spiperone (0.2, 0.4 and 0.8 mg/kg), (but not a D1 antagonist) suppressed QBA in a dose- and time- dependent manner. The facilitatory effects of APO (1.4 mg/kg) were completely blocked following pretreatment with spiperone (0.2 mg/kg) 10 min prior to APO injections. The results indicate that DA regulates QBA and that this effect is mediated through D2 receptors. **[Supported by NIH grant NS07941-20].**

433.6

DIFFERENTIAL EFFECTS OF SELECTIVE DOPAMINE AGONISTS AND ANTAGONISTS IN RANDOM AND INBRED MICE STRAINS. M. A. Libonati* and V. L. Coffin. Schering-Plough Research, Bloomfield, N.J. 07003

The effects of SKF 38393 (a selective D1 agonist) and SCH 23390 (a selective D1 antagonist) on grooming behavior were compared among a random-bred (CF-1) and three inbred-derived (A/J, DBA/2J, C57BL/ 6J) mice strains. In A/J mice, SKF 38393 enhanced grooming (3.0-30.0 mg/kg i.p.) while SCH 23390 had no effect over a 100-fold dose range (0.003-0.3 mg/kg i.p) except to decrease grooming at the highest dose tested (0.3 mg/kg i.p.). The other inbred strains, C57BL/6J and DBA/2J, showed relatively no effect from either SKF 38393 or SCH 23390 on grooming and the random-bred CF-1 strain showed enhanced grooming from both SKF 38393 (1.0-30.0 mg/kg i.p.) and SCH 23390 (0.003-0.3mg/kg i.p.). Subsequent studies with the inbred A/J strain mice showed stereospecificity for the enantiomers of SKF 38393 on grooming behavior. The (+)enantiomer increased the grooming response (10.0-30.0 mg/kg i.p.) and at higher doses the (-)enantiomer decreased grooming (60.0-100.0 mg/kg i.p.). Selective D2 agonists, LY 171555 and (+/-)PPHT HCl, produced large decreases in grooming in A/J mice, as did the mixed D1/D2 agonist apomorphine. Similarly, selective D1 and D2 antagonists decreased grooming in A/J strain mice. The selective D1 antagonist SCH 39166 reduced grooming at 0.1 &0.3 mg/kg i.p., while haloperidol, a selective D2 antagonist and the atypical dopamine antagonist clozapine attenuated grooming at higher doses (1.0-3.0 and 10.0-30.0 mg/kg i.p., respectively). These results demonstrate grooming behavior was selectively enhanced by D1 agonists in A/J strain mice; all other dopaminergic drugs produced decreases in grooming behavior. The findings suggest measurement of grooming response in A/J strain mice might be a useful model to predict selective D1 agonist activity in novel compounds.

433.7

BEHAVIORAL ANALYSIS OF D1 RECEPTOR ANTAGONISM: INTERACTION WITH D-AMPHETAMINE. M. Haney, J.W. Tidey, J.A. Vivian, K.A. Miczek. Dept. Psychology, Tufts Univ., Medford, MA 02155.

Dopamine's role in the behavioral properties of psychomotor stimulants has primarily been investigated with D2 receptor selective agents. We examined the intrinsic and pharmacological antagonistic role of the D1 receptor subtype in several salient behavioral patterns of male mice. Intruder-directed aggressive behavior, motor activity, and conditioned performance (multiple FI10 FR30 schedule) were concurrently assessed. Mice received SCH23390, a D1 antagonist, (0.01-0.3 mg/kg ip) in the presence of either saline or *d*-amphetamine (d-AMPH, 0.1-6.0 mg/kg ip). Conditioned performance was measured for 60 min post-injection. After ca. 22 min in the operant chamber, mice were returned to their home cage, where they exhibited aggressive behavior toward an intruder for ca. 5 min. Thereafter, the mice completed the remainder of the conditioning session. Each dose of SCH23390 decreased FR and FI response rates. Aggressive but not locomotor behavior was also decreased by D1 antagonism. The suppressive effects of SCH23390 on operant and aggressive responding were reversed with increasing doses of d-AMPH. At low d-AMPH doses, SCH23390 attenuated aggressive behavior. SCH23390 blocked the disruption of aggressive behavior by higher d-AMPH doses. The suppressive effects of higher d-AMPH doses on schedule-controlled behavior were not reversed by SCH23390. Therefore, D1 receptor antagonism exerts selective effects on the behavioral properties of psychomotor stimulant drugs. The intrinsic effects of SCH23390 suggest that the D1 receptor tonically modulates certain high rate behaviors.

433.8

WITHDRAWN

433.9

D-1/D-2 DOPAMINE RECEPTORS INVOLVEMENT IN LOCOMOTOR RECOVERY OF RESERPINIZED MICE ELICITED BY FCE 23884.

M. Buonamici*, M.A. Cervini*, R. Maj*, A.C. Rossi, R. Roncucci* and R.G. Fariello R&D., Farmitalia Carlo Erba - Erbamont Group, CNS Dept, 20014 Nerviano, Italy

FCE 23884 is an ergoline compound with the unique characteristic of acting as an antidopaminergic agent in normal animals, turning, at the same doses into a strong dopamine (DA)-ergic agent in "denervated" animals. The compound proved to be mainly a D-1 agonist in the model of 6-OHDA lesioned rats. In mice FCE 23884, injected 18 h after reserpine, stimulated locomotor activity in a dose-dependent manner. Also the selective DA-ergic D-1 (SKF 38393) and D-2 (LY 171555) agonists restored locomotion in 18 h reserpinized mice in a dose-dependent manner, but to a lesser extent than FCE 23884.

It is well known that: 1) D-2 receptors are involved in the mechanisms of locomotion and rearing (Starr and Starr, Neuropharmacology, 25, N.5: 455, 1986); 2) this D-2 modulated-activity can be initiated by D-1 stimulation (Molloy and Waddington, Eur. J. Pharmac., 108: 305, 1985) and 3) behavioral stimulation is induced by separate DA-ergic D-1 and D-2 receptor sites in long-term reserpine treated rats (Arnt, Eur. J. Pharmac., 113: 79, 1985).

Interaction studies were performed to better understand FCE 23884 activity in 18 h reserpinized mice. Pretreatment with the D-1 antagonist SCH 23390 almost completely antagonized FCE 23884 (83%) and SKF 38393 (95%) activity, while it proved inactive on LY 171555 effect. Pretreatment with the D-2 antagonist L-sulpiride completely antagonized LY 171555 activity and largely antagonized both FCE 23884 (60%) and SKF 38393 (77%) effects. These results suggest that in this reserpine model: 1) both D-1 and D-2 DA-ergic agonists restore locomotor activity; 2) D-1 blockade does not influence the D-2 mediated locomotor activity; 3) D-2 blockade reduces the recovery of motility induced by putative selective D-1 agonists.

433.10

LOCOMOTOR EFFECTS OF SELECTIVE DOPAMINE AGONISTS ADMINISTERED INTO THE NUCLEUS ACCUMBENS. W. D. Essman[1], S. M. Nair*[2], P. McGonigle[2] and I. Lucki[1,2]. Depts. of Psychiatry[1] and Pharmacology[2], Univ. of Pennsylvania, Philadelphia, PA, 19104.

Both synergistic and opposing functional effects have been described between the dopamine D1 and D2 receptors. Neurophysiological and biochemical investigations of these interactions have focused on the actions of dopaminergic compounds in discrete brain regions, whereas most behavioral interaction studies have involved peripheral administration of D1 and D2 agonists and antagonists. The present experiments measured the locomotor activity engendered by selective D1 and D2 agonists administered into one of the major DA projection areas, the nucleus accumbens (Acb). The interaction between these receptor types in producing a behavioral effect mediated through a specific brain region was examined.

Male Sprague-Dawley rats were stereotaxically implanted bilaterally with guide cannulae aimed at the Acb. After recovery, the effects of bilateral intra-accumbens microinjections of several doses of d-amphetamine, the D2 agonist quinpirole, or the D1 agonist SKF 38393 on locomotor activity were assessed in computer-assisted automated activity chambers.

Centrally administered d-amphetamine engendered a dose-dependent increase in locomotor activity. Quinpirole did not elicit locomotion at low doses, but did increase activity in some animals at high doses. SKF 38393 increased locomotor behavior at intermediate, but not high, doses. An increase in locomotion was observed following central administration of a low dose of quinpirole when the subjects were given a concurrent peripheral injection of SKF 38393. Coadministration of both quinpirole and SKF 38393 into the Acb elicited an increase in activity greater than either agonist alone. These data suggest that the actions of the DA receptor subtypes in the Acb act synergistically to engender locomotion.

433.11

THE EFFECT OF CLOZAPINE ON STIMULUS FILTERING / SELECTIVE ATTENTION IN RATS. L.A. Dunn, R.J. Scibilia, G. Schutrum*, C.D. Kilts. Dept. of Psychiatry, Duke Univ. Med. Ctr., Durham, NC 27710.

Latent inhibition (LI) of a conditioned response is a behavioral index of the stimulus filtering aspect of selective attention which is commonly observed to be defective in schizophrenia. LI is enhanced by anti-psychotic drugs and is attenuated by amphetamine treatment in rats. Clozapine is an atypical antipsychotic drug which does not cause extra-pyramidal symptoms or tardive dyskinesia. Clozapine is also atypical in its effects in various animal behavioral models of anti-psychotic drug action. We have evaluated the effect of clozapine on LI using an established paradigm (Christison et al., Biol. Psychiat., 23:746-749, 1988). The effect of clozapine (10 mg/kg, i.p., 7 days) on LI as a function of number (0, 10, 20, 40) of stimulus preexposures was examined. Unlike haloperidol, no effect of clozapine on LI was observed at any preexposure level. In an effort to approximate clinical dosing regimens, the effect of clozapine on LI was assessed at 0 and 20 preexposures using a t.i.d. dose of 5mg/kg (i.p.) administered for 7 days. Clozapine significantly decreased LI, an effect usually seen with amphetamine. These results suggest that the therapeutic effects of clozapine are mediated by unique actions relative to typical antipsychotic drugs. (Supported by the Scottish Rite Foundation)

433.12

PHARMACOLOGICAL ANALYSIS OF THE ENHANCED GROOMING RESPONSE ELICITED BY THE D1 DOPAMINE RECEPTOR AGONIST SKF 38393 IN THE RAT. R.J. Brooderson, S.R. Wachtel, and F.J. White. Depts. of Pharmacol. and Psychiat., CCN Program, Wayne State Univ. Sch. of Med. and Neuropsychopharmacol. Lab., Lafayette Clinic, Detroit, MI 48207.

The pharmacology of the enhanced grooming response elicited by dopamine (DA) agonists in rats was investigated. The amount of time spent grooming was measured continuously for 30 minutes following drug administration to provide a quantitative measure of the drug-induced behavior. The D1 DA agonist SKF 38393 dose dependently (0.5-16 mg/kg, s.c.) elicited an increase in the amount of time spent grooming. The SKF 38393-induced grooming demonstrated stereoselectivity for R-SKF 38393. Unlike SKF 38393 the peripheral D1 agonist fenoldopam (SKF 82526) failed to elicit a change in grooming. The SKF 38393-induced increase in grooming was attenuated by the D1 selective antagonist SCH 23390 (0.05 mg/kg, s.c.), in a competitive manner. The D2 selective antagonist eticlopride (0.05 mg/kg, s.c.) produced a noncompetitive blockade of SKF 38393-induced grooming. When eticlopride was coadminstered with the mixed D1/D2 agonist apomorphine, an increase in grooming behavior similar to the SKF 38393-induced effect was observed. Pretreatment with SCH 23390 (0.5 mg/kg), to protect the D1 receptor from inactivation by the irreversible receptor inactivator EEDQ (8 mg/kg, I.P.), prevented the attenuation of SKF 38393-induced grooming. However, in EEDQ pretreated rats without D2 receptor protection, eticlopride (0.2 mg/kg, s.c.) still prevented the SKF 38393-induced grooming response suggesting this effect may not be due to D2 dopamine receptor antagonism. These results demonstrate that excessive grooming elicited by dopamine agonists is a behavior specifically associated with central D1 DA receptor activation and is not mediated by D2 DA receptors. This research was supported by DA 04093, MH 40832 and by the State of Michigan.

433.13

THREE CHOICE DRUG DISCRIMINATION: FAILURE TO OBSERVE POST-HALOPERIDOL REBOUND. W. F. Caul, J. R. Jones, S. M. Murphy* and T. A. Schmidt*. Dept. of Psychology, Vanderbilt University, Nashville, TN 37240.

Caul, Jones, and Barrett (1989) have reported that a single injection of 1 mg/kg haloperidol (HD) 23 hr prior to testing increased the percent of responses made on the amphetamine-appropriate lever and decreased the total number of responses made by rats previously trained to discriminate amphetamine (AM) from distilled water (DW). The experiment reported here was designed to evaluate the feasibility of using an appetitive three-choice drug-discrimination task with rats to further evaluate such rebound responding based, presumably, on a continuum of dopaminergic activity. Rats were trained to discriminate among .75 mg/kg AM, DW, and .02 mg/kg HD. On the 13th exposure to AM during training, the animals responded 84% on the AM lever, 9% on the DW lever and 7% on the HD lever. When given DW, they responded 7% on the AM lever, 67% on the DW lever, and 26% on the HD lever. The 13th exposure to HD produced 5% responding on the AM lever, 19% on the DW lever and 76% on the HD lever. Following the exact procedures used in our previous experiment with a two-lever task, responding was evaluated 23 hrs following an injection of either 1 mg/kg HD or DW. In contrast to the results of the two-lever study, there was no difference in percent amphetamine-lever responding between the two groups although total responding was reduced in the HD-injected group. By repeating the injection and test procedure on each of two additional days, extinction-induced changes in choice responding were observed.

433.14

DOPAMINE D1 AND D2 AGONISTS AND ANTAGONISTS PRODUCE TURNING WHEN INJECTED UNILATERALLY INTO THE NUCLEUS ACCUMBENS. C. MESSIER, O. MRABET* and C. DESTRADE. Psychophysiologie Lab., URA CNRS n°339, U. Bordeaux I, 33405 Talence, FRANCE.

We have previously shown that intra-accumbens microinjection of the non-specific DA agonist apomorphine and the non-specific DA antagonist haloperidol produced respectively contra- and ipsilateral turning (C.R. Acad. Sci. (Paris), **309** (III), 77-82, 1989). We have extended these findings by examining the effect on behavior of intra-accumbens injection of the specific D1 agonist SKF38393, the D2 agonist LY171555, the specific D1 antagonist SCH23390 and the D2 antagonist metoclopramide. Mice were implanted with a cannula in the nucleus accumbens (from bregma AP= +1.7 mm; L= 1.6 mm and 2.9 mm below the surface of the skull). Ten days later, mice were placed in glass bowls and the number and the direction (ipsi- or contralateral to the injection site) of complete rotations were recorded. SKF38393 (3.5 µg) did not produce any change in the direction of locomotion. LY171555 (10 µg) produced contralateral turning which was potentiated when SKF38393 (3.5 µg) was injected together with LY171555. SCH23390 (5 µg) and metoclopramide (30 µg) produced ipsilateral turning which was not as important as the ipsilateral turning produced by haloperidol (5 µg). These results suggest that dopaminergic receptors in the nucleus accumbens also contribute to the direction of locomotion and that D1 and D2 receptors may act synergistically to produce this effect.

433.15

LOCALIZATION OF BEHAVIORAL EFFECTS OF DOPAMINE AGONISTS WITHIN THE STRIATUM OF 6-OHDA LESIONED RATS. H. E. Criswell, D. C. Lado, G. E. Duncan, T.J. McCown, U. B. Schambra, G. R. R.A. Mueller, G.R. Breese. University of North Carolina, Chapel Hill, N.C. 27599-2750.

Rats, neonatally lesioned with 6-OHDA, were sensitized by repeated systemic administration of SKF-38393 (Criswell et al., Brain Res., in press). Subsequently, this D_1 agonist (3 ug) or a D_2 agonist (0.3 ug of Quinpirole) was micro-injected bilaterally into the ventrolateral, dorsolateral, ventromedial or central caudate nucleus. All rats showed an increased behavioral response when compared to either unlesioned or saline treated controls. Only microinjections into the ventrolateral caudate evoked paw licking or taffy pulling. Other behaviors were less site specific. The behavioral responses elicited by D_1 and D_2 agonists were similar. Thus, the site of the microinjection was more important in determining the type of behavior observed than was the receptor subtype activated. Density of D_1-receptors in the caudate was measured with ^{125}I-SCH23390 autoradiography and densitometry. D_1-receptor density was higher in the ventrolateral caudate than in the other quadrants. Supported by HD-03110, HD-23082, NS-21345.

433.16

A PRIMATE MODEL OF HUNTINGTON'S DISEASE: DOPAMINERGIC INVOLVEMENT IN DYSKINESIAS. O. Isacson, J.M. Schumacher, M.S. Fiandaca, P. Hantraye*, B.M. Madras, R.S. Spealman and E.D. Bird. Dept. Neurology and Neurosurgery, Harvard Med. Sch., McLean Hospital, Belmont MA 02178, Massachusetts General Hospital, Boston, MA, NERPRC, Division of Behavioral Biology, Harvard Med. Sch., U. Mass and SHJF, France.

Excitotoxic lesions of the caudate-putamen complex (CP) in non-human primates produce an animal model for symptoms and palliative treatments of Huntington's disease (HD). Following dopamine agonist administration animals with unilateral caudate-putamen lesions show abnormal movements including dystonia, oro-facial dyskinesia, head-dyskinesia, rotation and chorea-like movements. We have investigated abnormal movements following excitotoxic CP lesions (ibotenic-acid and quinolinic acid) in baboons and cynomolgus monkeys and the ability of various pharmacological agents acting on the dopaminergic system to elicit dyskinesias. Following administration of the D1/D2 dopamine receptor agonist apomorphine (1mg/kg i.m.) dyskinesias were elicited only in lesioned animals. Comparison of movements produced by apomorphine with the selective D2 receptor agonist PHNO (0.01-0.1mg/kg) demonstrated that both drugs resulted in increased locomotor activity. The type of movements elicited by PHNO was predominantely oro-facial and dose-dependent, while apomorphine at doses > 1mg/kg resulted in a wide spectrum of motor activity including chorea-like movements. The selective dopamine uptake inhibitor GBR 12909 (0.3-5.6 mg/kg) caused yet another pattern of locomotor hyperactivity of lesser magnitude.

Our results indicate the differential involvement of dopamine-receptor activation in dyskinesias produced by selective excitotoxic caudate-putamen lesions in the non-human primate with HD-like pathology.

433.17

CLOZAPINE EXHIBITS D1 DOPAMINE ANTAGONIST EFFECTS IN VIVO AS ASSESSED WITH [^{14}C]-2-DEOXYGLUCOSE AUTORADIOGRAPHY. Catherine A. Leslie (1) and Joel M. Trugman (2), Departments of Behavioral Medicine and Psychiatry (1) and Neurology (2), University of Virginia School of Medicine, Charlottesville, VA 22908.

[^{14}C]-2-Deoxyglucose (2-DG) autoradiographic studies of dopamine (DA) agonist-induced turning in rats with unilateral 6-hydroxydopamine lesions of the nigrostriatal pathway have shown that D1 agonists (SKF 38393) markedly increase regional cerebral glucose utilization (RCGU) in the substantia nigra pars reticulata (SNr) ipsilateral to the lesion whereas D2 agonists (LY 171555) do not. Furthermore, RCGU increases in the SNr induced by a mixed agonist (L-Dopa) are completely blocked by a selective D1 antagonist (SCH 23390) but only partially attenuated (by about 50%) by a selective D2 antagonist (eticlopride).

This suggests that the ability of a drug to block RCGU increases induced by L-Dopa can be used to assess D1 antagonist effects in vivo. We tested the effects of the "atypical" antipsychotic clozapine in this model. Rats with unilateral lesions were pretreated with clozapine (10 mg/kg) 30 minutes prior to the administration of L-Dopa (25 mg/kg) and [^{14}C]-2-DG. Compared to rats which received L-Dopa alone, clozapine pretreatment attenuated contralateral rotation and completely blocked RCGU increases in the SNr. The data demonstrate D1 antagonist effects of clozapine in vivo and suggest that the "atypical" neuroleptic profile of this drug may be mediated in part by D1 receptor antagonism.

433.18

RELATIONSHIP BETWEEN BEHAVIOURAL ACTIVATION AND ADENYLATE CYCLASE ACTIVITY INDUCED BY THE DOPAMINE D$_1$ RECEPTOR AGONISTS SK&F 80723, 83565 AND 83959. D.C. Rogers*, A.J. Hunter* and R.G. Hill. Smith-Kline Beecham Pharmaceuticals, The Frythe, Welwyn, Herts., U.K.

Previous studies with the partial agonist SK&F 38393 have suggested that stimulation of the D_1 receptor alone is not sufficient for the expression of dopamine-mediated behaviour, and that concomitant stimulation of both D_1 and D_2 receptor related mechanisms is necessary (White *et al*, *Pharmacol. Biochem. Behav.*, 30:189, 1988). The development of the novel D_1 receptor agonists SK&F 80723, SK&F 83565 and SK&F 83959 has enabled us to investigate whether the effects of SK&F 38393 are characteristic of all D_1 agonists.

Following habituation to the activity cages, rats were injected s.c. with either vehicle, 0.1mg/kg or 1.0mg/kg of SK&F 80723, 83565 or 83959. The locomotor activity (LMA) of the rats was recorded for 60 min. During this time, the behaviour of each animal was monitored for 1 min in each 5 min period. Adenylate cyclase activity was assessed with the determination of cAMP accumulation in rat striatum *in vitro*.

SK&F 80723 produced a significant increase in LMA [log.counts $F(2,21)=12.13$, $p<0.001$], displayed adenylate cyclase activity and had a significant effect on the duration of grooming behaviour [$F(2,21=3.90$, $p<0.05$]. SK&F 83565 and SK&F 83959 both induced significant increases in grooming [$F(2,21)=18.01$, $p<0.001$] and [$F(2,21)=7.97$, $p<0.01$] respectively, in the absence of significant increases in LMA or cyclase activity.

These results suggest that the characteristic D_1 activated grooming behaviour is not mediated via adenylate cyclase and that LMA may indeed be stimulated by D1 receptor activation alone.

433.19

RADIOPROTECTIVE EFFICACY OF 17β-ESTRADIOL IN MICE. M. Miernicki, H.D. Davis*, and M.R. Landauer*. Behavioral Sciences Department, Armed Forces Radiobiology Research Institute, Bethesda, MD 20814-5145.

Recent studies indicate that estradiol benzoate and other estrogen derivatives increase survival when administered to mice following exposure to γ radiation. This study was designed to determine the radioprotective efficacy and behavioral toxicity of 17β-estradiol (E2) when administered prior to irradiation.

Ten days before irradiation, gonadally intact male CD2F1 mice (N=16/group) were given a single IM injection of either E2 (0.16 mg/0.1 ml sesame oil), the sesame oil vehicle (OIL; 0.1 ml), or were not injected (CONT). On the day of irradiation, mice were exposed to 8.5 Gy 18 MVp Bremsstrahlung in 3.0 μsec pulses delivered at a dose rate of 1.0 Gy/min.

Significantly more E2 treated mice survived for 30 days (75%) than either OIL (50%) or CONT (25%) animals. In separate groups of identically treated, but nonirradiated mice, a single E2 injection resulted in decreased spontaneous locomotor activity within 3 hrs of administration. These results suggest that the radioprotective effect of E2 is accompanied by concurrent behavioral toxicity as measured by decrements in locomotor activity. We are presently investigating whether the radioprotection produced by estrogenic hormones can be maintained while attenuating the behavioral side-effects.

433.20

Determinants of Behavioral Effects of Corticotropin Releasing Hormone in the Rhesus Monkey J. R. Glowa Biopsychology Unit, CNE, NIMH, Bethesda, MD 20892,

Previous studies have determined the dose-effect and time course for the behavioral effects of corticotropin releasing hormone (CRH) on FR food-maintained responding in macaques. Doses of 0.03 - 10 ug/kg decrease responding, with the effects of lower doses requiring as much as an hr before manifestation, while those of higher doses occur within minutes. The current studies show: 1) repeated dosing with as little as 3 ug/kg results in a sustained effect on food maintained responding lasting well beyond the period of dosing; 2) responding maintained by the termination of stimuli associated with noxious stimuli is less sensitive to the rate-decreasing effects of CRH than is food-maintained responding, dose-effect functions in the same animals as those assessed for food lie to the right of the food-maintained dose-effect functions; and 3) the effects of CRH on food-maintained responding seem relatively unaffected by the familiarity of the context in which the macaques were dosed, dose-effect functions obtained in isolation chambers vary little from those obtained in the home cage. These results suggest that food-maintained responding serves as a reliable, yet sensitive baseline to assess the behavioral effects of CRH, and suggests treatment approaches should be directed at this end point.

433.21

ASSESSMENT OF DSIP ON SCHEDULE-CONTROLLED BEHAVIOR IN RATS. R.F. Genovese, X-C.M. Lu*, and D.L. Yourick, Department of Medical Neurosciences, Walter Reed Army Institute of Research, Washington, DC 20307.

Delta sleep-inducing peptide (DSIP) is a nonapeptide originally notable for its somnogenic properties. Subsequently, DSIP has been shown to have a variety of effects not typically associated with sleep, including naloxone-sensitive antinociception and attenuation of thermic and locomotor effects of amphetamine. We further investigated DSIP using a multiple fixed-ratio, fixed-interval schedule of reinforcement (FR 20, EXT 15", FI 2', EXT 15"). The schedule produced distinct rates and patterns of responding in all rats. Specifically, response rates in the FR > FI > EXTs. Additionally, quarter-life values in the FI component were typically > 60%. DSIP administered ICV (3.2-100.0 μg) (n=6) and SC (0.32-5.6 mg/kg) (n=6) failed to produce substantial effects on response rate or pattern in any of the schedule components at any dose, regardless of the route of administration. These results are surprising and further research is in progress to assess the effects of DSIP administered in combination with d-amphetamine.

434.1

MODULATORY EFFECTS OF CARBACHOL ON RAT VISUAL CORTICAL NEURONS IN VITRO. T. Murakoshi. Laboratory of Neurobiology, The Rockefeller University, New York, NY 10021.

To explore the role of acetylcholine in rat visual cortex, the cholinergic agonist, carbachol (CCh), was applied to neurons in cortical slices. Intracellular recordings were made in coronal slices of cortex, and CCh was applied either by perfusion (30 uM) or focally by pressure puffs (30 mM, 0.05-0.5 sec). Several effects were observed. The most common effect was slow depolarization with no change or with a slight decrease in membrane conductance (in 48 out of 56 cells). The remaining cells showed slow hyperpolarization with a conductance increase. Both types of response were likely the result of a direct effect of CCh on the membrane because they persisted when synaptic transmission was blocked by TTX. A marked effect of CCh application was an increase in the rate of firing in response to depolarizing pulses. This was due to a shortening of the time course of repolarization after each spike. A similar observation has been reported in CNS neurons (Cole, A.E. & Nicoll, R.A., J. Physiol. , 352:173, 1984). Both the changes in membrane potential and firing rate were blocked by atropine (0.3 uM). CCh also affected synaptic transmission. It attenuated both epsps and ipsps evoked by stimulating the underlying white matter, particularly the slow ipsp. The net result of the various effects of cholinergic modulation appeared therefore to be an increase in excitability to repetitive afferent stimuli. (supported by the NEI US-Japan Exchange Scientist Program and by NIH grant EY05253 and NS22789)

434.2

MUSCARINIC AGENT INDUCED NEURONAL RESPONSES IN RAT CEREBRAL CORTEX. Y. Lin and J.W. Phillis. Dept. of Physiology, Wayne State University School of Medicine, Detroit, MI 48201.

Recording of neuronal activity and iontophoretic application of various agents were accomplished with seven-barrelled micropipettes in anaesthetized rats to evaluate muscarinic agonist action on cortical neuronal activities.

Oxotremorine-M excited spontaneously active deep cerebral cortical neurons. No excitation was observed with oxotremorine or McN-A-343.

Effect of muscarinic agonists on evoked neuronal discharges **

agent	glutamate evoked discharges			Ach evoked discharges	
	inhibited	inh./enh.*	enhanced	inhibited	inh./enh.*
oxotremorine	25/55	19/55	11/55	12/15	3/15
McN-A-343	20/37	11/37	6/37	11/11	0/11
oxotremorine-M	59/71	8/71	4/71	19/19	0/19

Blockade of agonists' inhibitory action on glutamate discharges **

agent	atropine		pirenzepine		gallamine		methoctramine	
	blocked	no	blocked	no	blocked	no	blocked	no
oxotremorine	3/4	1/4	7/12	5/12	1/5	4/5	----	----
oxotremorine-M	12/12	0/12	2/7	5/7	5/5	0/5	5/7	2/7

** numbers represent cells tested; * inh=inhibited, enh=enhanced; no=unblocked.

Conclusion: cortical muscarinic receptors mediate multiple synaptic actions. The inhibitory action appears to be non-selective since both glutamate and Ach evoked discharges were supressed. Following the inhibition, an enhancement of glutamate evoked discharges (for as long as 2 hours) was induced in some neurons. Both M_1 and M_2 antagonists blocked the excitation by oxotremorine-M of spontaneously activite neurons.

434.3

CHOLINERGIC MODULATION OF E-S COUPLING IN CA1 PYRAMIDAL CELLS OF THE RAT HIPPOCAMPUS.

M.K. Meffert, G.A. Cohen and D.V. Madison. *Dept. of Molecular and Cellular Physiology, Stanford Univ. Sch. of Med., Stanford, CA 94305.*

We have studied the effects of carbachol at the Schaffer collateral-CA1 synapse of the rat hippocampus. Carbachol (20 or 50uM) initially depressed the evoked EPSP field potential (~90% inhibition) and the population spike. However, the population spike recovered to near or above its baseline amplitude within a few minutes in the continued presence of carbachol, even though the epsp remained inhibited. Thus, it seemed apparent that the coupling between the epsp and the population spike (E-S coupling) was enhanced. Comparison of input/output curves showed that population spikes could be elicited by lower stimulus voltages than in control conditions and that maximal population spike amplitudes were reached at much lower stimulus strengths.

Effects of carbachol on E-S coupling may bear on the interpretation of previous results. For example, adenosine blockade of synaptic transmission appears to be inhibited by carbachol (PNAS, 84:3467, 1987; Science, 239:1428, 1988). Like these previous reports, we find that adenosine, in the presence of carbachol, fails to inhibit population spikes elicited by near maximal stimulation. However, we believe that this inhibition is an indirect result of carbachol shifting the input/output curve to the left. Because of this shift, near maximal stimulation becomes supra-maximal in the presence of carbachol such that inhibitory influences on the population spike are less effective. When the stimulus strength is readjusted to be sub-maximal in the presence of carbachol, it is revealed that adenosine still potently inhibits the population spike.

Perhaps the most intriguing aspect of these findings is that carbachol increases E-S coupling to the extent that stimulation produces a full-amplitude population spike even though the epsp is almost completely blocked. We are investigating the mechanisms underlying this phenomenon. *D.V.M. is a Lucille P. Markey Scholar and this work was supported in part by a grant from the Lucille P. Markey Charitable Trust. G.A.C. is a Howard Hughes Medical Institute Predoctoral Fellow.*

434.4

LITHIUM POTENTIATES CHOLINERGIC PRESYNAPTIC INHIBITION IN AREA CA1 OF THE RAT HIPPOCAMPUS.

G.A. Cohen and D.V. Madison. *Dept. of Molecular and Cellular Physiology, Stanford Univ. Sch. of Med., Stanford, CA 94305.*

It has been shown that activation of muscarinic receptors in hippocampus mediates an inhibition of excitatory transmission by a presynaptic mechanism. In agreement with previous findings, carbachol causes a marked inhibition of epsp field potentials recorded in stratum radiatum, or in intracellularly recorded epsp's. To study the nature of this inhibitory effect we have used whole cell voltage clamp techniques in submerged, 500 um thick rat hippocampal slices to study the effect of carbachol in minimally stimulated excitatory postsynaptic currents (epsc's). Because of the greatly reduced noise inherent in whole cell recording, it is possible to detect epsc's produced by single presynaptic axons. Minimal stimulation to the Schaffer collaterals produces very small epsc's resulting from the activation of a very small number or single presynaptic axons. Stimulus strength was adjusted to give an epsc failure rate of between 10 and 50%. Application of carbachol caused an increase in the frequency of epsc failure. This is strong evidence confirming the presynaptic nature of carbachol's inhibition of excitatory transmission.

To test if the mechanism of carbachol's inhibition of synaptic transmission might involve phosphoinositide (PI) turnover, we tested the effects of lithium, a blocker of the PI turnover cycle. LiCl alone (2mM) caused a slight inhibition in the slope of the evoked field epsp and the amplitude of the population spike, but greatly potentiated carbachol's (1uM) inhibition of the epsp and inhibition of the evoked population spike amplitude. In whole cell recordings, LiCl (2mM) had no detectable effect alone, but markedly potentiated the ability of carbachol to increase the frequency of failures of very small epsc's evoked by minimal stimulation. This is evidence that lithium may act on excitatory presynaptic terminals and may show a physiologically important action of this psychiatrically useful agent. *D.V.M. is a Lucille P. Markey Scholar and this work was supported in part by a grant from the Lucille P.Markey Charitable Trust. G.A.C. is a Howard Hughes Medical Institute Predoctoral Fellow.*

434.5

VOLTAGE-CLAMP ANALYSIS OF CHOLINERGIC ACTION IN THE BASOLATERAL AMYGDALA. M.D. Womble and H.C. Moises. Dept. of Physiology, Univ. of Michigan, Ann Arbor, MI 48109.

Previously, we showed that the cholinergic agonist carbachol depolarizes neurons of the basolateral amygdala (BLA) and reduces both accommodation and the late afterhyperpolarization (AHP) (Washburn & Moises, Neurosci. Abstr. 15:193, 1989). We have also identified many of the membrane currents in BLA neurons (Moises and Womble, this volume). In this study, we used the single electrode voltage-clamp technique to identify those currents in BLA neurons of rat brain slices which were sensitive to cholinergic modulation. M-current (Im) was identified as a slowly decaying, voltage-sensitive current, inactivating during hyperpolarizing steps (1s, 15 mV) from a holding potential (Vh) of -40 mV. Carbachol (10-40 uM) blocked Im and increased input resistance at depolarized levels. The slow AHP following an action potential was blocked by carbachol, as was the underlying Iahp. The action of carbachol was accompanied by an inward shift in holding current and an increase in input resistance even at those potentials (-70 mV) where Im and Iahp were not activated. This indicates that carbachol also acts to block a resting 'leak' conductance. All the actions of carbachol were blocked by atropine (1 uM). Thus, block of the leak current by carbachol may underlie neuronal depolarization, while the loss of accommodation may be the result of Im and Iahp inhibition. (Supported by NIDA grant DA03365.)

434.6

THE MUSCARINIC AGONIST BETHANECHOL INITIATES HYPERPOLARIZATION AND DEPOLARIZATION OF MUDPUPPY INTRACARDIAC NEURONS. L.M. Konopka and R.L. Parsons. University of Vermont College of Medicine, Burlington, VT 05405.

Previously it was shown that muscarinic activation results in the hyperpolarization of mudpuppy parasympathetic postganglionic neurons by activation of a potassium conductance (Hartzell et al., J. Physiol., 271, 1977). Our recent experiments demonstrate that the muscarinic agonist bethanechol can elicit a biphasic response: hyperpolarization followed by depolarization or depolarization, which preceded and followed the hyperpolarization. The average amplitudes of the hyperpolarization and depolarization, measured at resting membrane potentials -40 to -65 mV under optimal agonist application conditions, were approximately 20 and 3 mV respectively. Both components of the response were eliminated by atropine (0.1-5 μM). Excitability of the neurons increased during the depolarizing component. In the presence of 2-4 mM Ba++, the bethanechol induced hyperpolarization was selectively blocked. In Ba++ the amplitude of the depolarization increased with membrane hyperpolarization and decreased with membrane depolarization. The reversal potential of the depolarization was approximately +5 mV suggesting the response was due to the activation of a nonselective cation conductance. The M1 specific muscarinic receptor blocker, pirenzepine, partially inhibited hyperpolarizations at concentrations $\leq$ 10 nM and blocked both responses at concentrations > 20 nM. The M2 specific muscarinic blocker AFDX116 selectively inhibited hyperpolarizations at concentrations $\leq$ 1 M. These results show that the bethanechol induced hyperpolarizations and depolarizations of mudpuppy cardiac neurons are mediated via two distinct muscarinic receptors. Supported by PHS grants NS 25973 and NS 23798.

434.7

EFFECT OF PHYSOSTIGMINE AND EXERCISE ON CHOLINEACETYLTRANSFERASE AND ACETYLCHOLINESTERASE ACTIVITIES IN BRAIN REGIONS OF RAT. S.M. Somani, S.R., Babu, S.P. Arneric, S.N. Dube and S. Evans, Department of Pharmacology and Medicine, Southern Illinois University School of Medicine, P.O. Box 19230, Springfield, Illinois 62794-9230 U.S.A.

This presentation addresses whether subacute physostigmine (Phy) and/or subacute exercise elicit any changes in the ChAT and AChE activities in four brain regions.

Male Sprague Dawley rats were divided into five groups: Gr. I, control; Gr. II, subacute treadmill exercise two weeks; Gr. III, subacute Phy (70 μg/kg, i.m. twice daily) for two weeks; Gr. IV, subacute Phy and single exercise 100% Vo_2 MAX;; Gr. V, subacute Phy + subacute exercise. Rats were sacrificed 24 hr after the last dose of Phy and/or the exercise. ChAT and AChE activities were determined by the method of Fonnum (1975). Corpus striatum showed ChAT activity 88%, 68%, 88% of control ($P<0.05$) in Gr. II, IV and V respectively and AChE activity 87% of control in Gr. IV. Cerebral Cortex showed ChAT activity 79% of control in Gr. V only. Brain stem showed ChAT activity 73%, 78%, 82% and 81% of control ($P<0.05$) in Group II, III, IV and V respectively and AChE activity 81 and 82% of control in Gr. II and III respectively. Hippocampus showed ChAT activity 72 and 73% of control ($P<0.05$) in Group IV and V respectively.

These results suggest that Phy or physical exercise or the combination of these two stressors depress ChAT and AChE activities in each brain region differently. (Supported by U.S. Army Contract No. DAMD 17-88-C-8024)

434.8

IS RELEASE OF ASCORBIC ACID IN CINGULATE CORTEX CONTROLLED BY ACETYLCHOLINE OR BY AFFECT? K. Mueller, Dept. Psychol., Texas Christian University, Fort Worth, TX 76129

Release of ascorbic acid in brain is still poorly understood. Surprisingly, in previous research, pilocarpine (a direct cholinergic agonist) increased the ascorbic acid signal in anterior cingulate cortex (ACC) by 95% even though ACC contains little acetylcholine. Did pilocarpine produce this effect by acting directly in ACC or by producing gastrointestinal distress which in turn produced affective changes? Since ACC is a component of the limbic system, affect may be relevant to changes in ascorbic acid. A series of experiments was conducted to begin to investigate this hypothesis. In all experiments, voltammetry in vivo using carbon paste electrodes monitored release of ascorbic acid from conscious behaving rats. The first experiment exploited the functional and anatomical differences between anterior and posterior cingulate cortex. In posterior cingulate cortex pilocarpine increased the ascorbic acid signal by only 55%. In another experiment, scopolamine (a cholinergic antagonist) virtually abolished the effect of pilocarpine in ACC. Finally, an equimolar dose of methscopolamine (which does not cross the blood-brain-barrier) produced a large decrease in the effect of pilocarpine in ACC. Thus, the effect of pilocarpine on ascorbic acid in ACC must be related in part to its gastrointestinal effects. Other research on the relationship between affect and ascorbic acid in ACC is in progress.

434.9

BASAL FOREBRAIN INVOLVEMENT IN TONE ELICITED NEURONAL RESPONSES IN RAT AUDITORY CORTEX. E.L. Moore, J.G. Townsel and H.K. Rucker. Department of Physiology, Meharry Medical College, Nashville, TN 37208.

The specific aim of this study was to examine the effects of basal forebrain microinfusion of muscimol, a GABA agonist, on high affinity choline uptake, spontaneous rates and tone elicited response profiles of neurons in auditory cortex. Baseline peristimulus time histograms (PSTHs) were constructed to best frequency tone pulses prior to muscimol (100 nmol/ul) infusion (1ul/1 minute) and compared to 4 experiment PSTHs constructed during the following 60 minutes. High affinity choline uptake (HAChU) was assayed using synaptosomes prepared from temporal cortex. HAChU was decreased 56% in muscimol injected animals vs. controls. The spontaneous discharge rate and tone elicited response decreased in 70% of the units after muscimol injection. In the remaining units, biphasic changes in spontaneous firing were accompanied by increases in tone elicited firing rates. These results were interpeted to suggest that basal forebrain cholinergic projections modulate neuronal discharge characteristics, particularly response gain, in sensory cortex.

434.10

LEARNING AND MEMORY DEFICITS IN RATS PRODUCED BY SELECTIVE BLOCKADE OF HIGH AFFINITY CHOLINE UPTAKE FOLLOWING SYSTEMIC ADMINISTRATION OF A-4. C.E. Tedford, V.B. Ruperto*, M.E. Grzelak *, M. Cohen-Winston* and L. C. Iorio. Schering-Plough Research, Bloomfield, New Jersey, 07003.

A-4, a bis-tertiary amine piperdine derivative of hemicholinium-3 (HC-3), was used to inhibit acetylcholine synthesis. Subcutaneous administration of A-4 produced a dose-dependent reduction in central high affinity choline transport (HAChT) measured ex vivo. Doses of 3, 10 and 30 mg/kg of A-4 produced a 23.7%, 70.0% and 76.7% reduction in hippocampal HAChT at 1 hr versus vehicle treated animals. Maximal reduction in hippocampal HAChT was seen within 30 min after sc administration of A-4. This reduction in HAChT was maintained for up to 24 hr after administration and HAChT returned to near control values 48 hr after administration of A-4. No changes in choline acetyltransferase or acetylcholinesterase enzyme activities were seen.

Behavioral studies in rats utilizing the passive avoidance response paradigm illustrated that animals treated with A-4 had impairments in 24 hr retention. A-4 (30 mg/kg) administered sc 30 min prior to training decreased step-through median latencies from 180 sec to approximately 45 sec. Furthermore, sc administration of A-4 immediately following the training session also resulted in impairments in 24 hr retention times. Analogous studies using intracerebroventricular administration of HC-3 or A-4 also produced impairments in 24 hr retention using the passive avoidance response paradigm. Thus, these studies indicate that selective blockade of central HAChT following systemic adminstration of A-4 results in learning and memory impairments.

434.11

SIMULTANEOUS FOREBRAIN AND PONTINE MICROINJECTIONS OF CARBACHOL SUPPRESS REM SLEEP. H.A.Baghdoyan, R.Lydic, T.M.Rutherford*, and S.G.Snyder*. Dept. of Anesthesia, Penn State Univ. College of Med., Hershey, PA 17033.

Converging data support the now widely held view that pontine cholinergic mechanisms play a key role in the generation of REM sleep. The basal forebrain has been suggested to be important for the generation of nonREM sleep, but the neurotransmitters involved in this putative basal forebrain function are unknown. The purpose of the present study is to begin examining the role of cholinoceptive forebrain systems in sleep cycle control by quantifying the effects of forebrain carbachol microinjections on sleep and on the REM sleep-like state produced by pontine microinjection of carbachol.

To date we have performed 22 carbachol (4μg/.25μl) and 18 saline (0.25μl) microinjections in 3 intact cats. Carbachol in the forebrain produced a 45% increase in waking (W), a 76% decrease in nonREM (S), and a 64% decrease in REM (D). Carbachol in the pons produced a 10% decrease in W, a 100% decrease in S, and a 387% increase in D. Simultaneous forebrain and pontine microinjections of carbachol produced a 44% increase in W, a 99% decrease in S, and a 163% increase in D. Thus, cholinoceptive forebrain mechanisms can enhance W, suppress S, and reduce the REM sleep enhancing effects of pontine carbachol administration.

Supported by grant MH45361 to HAB.

434.12

NORADRENERGIC/ADRENERGIC INPUT TO CHOLINERGIC FOREBRAIN PROJECTION NEURONS. L. Zaborszky, W.E. Cullinan and L. Heimer. University of Virginia Health Science Ctr., Charlottesville, VA 22901.

We have reported previously (Zaborszky et al., 1987; Neuroscience, Suppl. S798) that tyrosine hydroxylase immunoreactive (TH) fibers in the forebrain surround cholinergic neurons and electron microscopic studies (Zaborszky et al., in press) confirmed the presence of synaptic contacts. Since TH is the first step in the catecholamine biosynthetic pathway, it is not clear whether such terminals represent dopaminergic and/or noradrenergic/adrenergic input.

In the present study, forebrain sections were stained with an antibody against dopamine-ß-hydroxylase (DBH), the enzyme present in noradrenergic/adrenergic neurons, in combination with ChAT (choline acetyltransferase) immunostaining, using the DAB/NiDAB double labeling technique. Adjacent sections were processed for TH/ChAT. DBH-positive fibers and terminals were in close proximity to cholinergic neurons throughout extensive forebrain areas, including the vertical and horizontal limb of the diagonal band nuclei, the sublenticular substantia innominata (SI), bed nucleus of the stria terminalis, ventral pallidum, and ventrolateral globus pallidus. It was confirmed in EM double-labeling studies that cholinergic neurons in the SI were contacted by DBH-positive axons through asymmetric synapses.

Based upon the differences in both the distribution pattern of TH/ChAT versus DBH/ChAT interactions, as well as the different morphological features of DBH versus TH-positive axon types, our study also revealed that cholinergic neurons in the ventromedial globus pallidus receive dopaminergic input. Supported by USPHS grants No. 23945 and 17743.

434.13

LOCUS COERULEUS PROJECTIONS TO THE ROSTRAL FOREBRAIN WITH SPECIAL REFERENCE TO INNERVATION OF CHOLINERGIC PROJECTION NEURONS.

W. E. Cullinan, R. Grzanna, and L. Zaborszky Univ. of Virginia Health Sciences Center, Charlottesville, VA, 22901, and The Johns Hopkins Univ. School of Medicine, Baltimore, MD, 21205.

Ascending projections from the locus coeruleus were demonstrated using the PHA-L anterograde tracing method, providing a precise view of the distribution and arborization pattern of locus coeruleus axons in the basal forebrain. To investigate whether these axons terminate on forebrain cholinergic projection neurons, PHA-L tracing and choline acetyltransferase (ChAT) immunocytochemistry were combined in double-labeling studies at both the light and electron microscopic levels using the nickel-enhanced DAB/DAB technique (Hsu and Soban, 1982). At the light microscopic level, locus coeruleus axons were detected in close apposition to cholinergic projection neurons within the medial septum/vertical limb of the diagonal band nucleus, horizontal limb of the diagonal band nucleus, and sublenticular substantia innominata. Results of experiments at the electron microscopic level demonstrated that cholinergic neurons are indeed contacted by locus coeruleus axons, and that an individual locus coeruleus axon terminal can establish synaptic contact with both cholinergic and non-cholinergic neuronal elements. In addition, immunofluorescence double-labeling confirmed that the vast majority of PHA-L labeled neurons at the injection site also contained the catecholamine synthesizing enzyme dopamine- ß-hydoxylase, thus, the present results indicate that noradrenergic input to forebrain cholinergic projection neurons originates in part from the locus coeruleus. Supported by USPHS Grants 23945 and 17743.

434.14

ULTRASTRUCTURAL FEATURES OF ACETYLCHOLINE (ACh) AXON TERMINALS IN ADULT RAT CEREBRAL CORTEX. D. Umbriaco, K.C. Watkins*, L. Descarries, C. Cozzari* and B. Hartman. CRSN (Département de physiologie), Université de Montréal, Montréal (CANADA); Istituto Biologia Cellulare CNR, Roma (ITALY), and Department of Psychiatry, University of Minnesota Medical School, Minneapolis (MN).

ACh axon terminals (varicosities) from the parietal (primary somatosensory) cortex of adult rat were examined by electron microscopic immunocytochemistry with a monoclonal antibody against purified rat brain choline acetyltransferase (MCAT-1C, Ka = $5x10^{10}$, from a series prepared in mouse). Initial fixation of the tissue was by perfusion with 2.5 % paraformaldehyde (PF) and 0.1-0.2% glutaraldehyde (pH 6.5), followed by PF alone (pH 8.5). Transverse vibratome sections were processed free-floating without detergent (dilution of primary antibody: 2 µg per ml), immunostained with the ABC method, osmicated, and flat embedded in Epon. Serial ultrathin sections were cut at right angles to the surface of vibratome sections. 140 immunostained varicosities from layer I were studied in 2 rats, being visualized in an average of 5.6 thin sections per varicosity. These varicosities measured 0.3 to 1.1 µm and averaged 0.5 µm in equivalent spherical diameter. Only 5% (n = 7), and a single one of 34 sectioned throughout, showed a synaptic membrane differentiation. The presence of a junction was not related to varicosity size. The junctional complexes were all symmetrical, and all contacts made on dendritic shafts. These results demonstrate that the ACh innervation of adult rat parietal cortex comprises a large proportion of non synaptic axonal varicosities, at least in layer I. They provide further evidence for the concept of "volume transmission", and support the general notion that, in the CNS, widely distributed projection systems issued from relatively small groups of nerve cell bodies are predominantly nonjunctional. [Supported by grants MT-3544 (MRC) and NS12311].

434.15

HUMAN BRAIN CORTICAL CHOLINERGIC INNERVATION.

Changiz Geula and Marsel Mesulam, Harvard U., Boston, MA

The peak density of histochemically demonstrated acetylcholinesterase (AChE)-rich, putatively cholinergic fibers was determined in 25 architectonic subregions of the cerebral cortex of five normal subjects.

Cortical areas were divided into 3 categories: 1) association, 2) primary sensorimotor and 3) paralimbic. All areas contained a dense net of AChE-rich fibers but with an individual pattern of lamination and density. Superficial layers generally contained a denser net of these fibers than the deeper layers. More fibers were encountered traveling vertical to the cortical surface as compared with fibers traveling horizontally.

The densest plexus of AChE-rich fibers was encountered in the paralimbic cortical areas (Mean peak density ± SD: 22.8±2.1) while the cortical association areas contained the lowest peak density (9.5±0.86). The primary sensorimotor areas contained an intermediate peak density of AChE-rich axons (14.3±1.5). The non-isocortical paralimbic zones (entorhinal cortex and the agranular and dysgranular components of orbitofrontal, temporopolar and insular cortex) contained a denser net of AChE-rich fibers (29.9± 2.5) than the immediately adjacent isocortical zones of the same paralimbic areas (17.0±1.5).

The results of this study are consistent with our previous observations in the monkey cortex and indicate that cortical cholinergic innervation in the human brain is much more intense in limbic and paralimbic areas than in association neocortex.

434.16

HUMAN NEOCORTICAL ACETYLCHOLINESTERASE NEURONS

Marsel Mesulam and Changiz Geula, Harvard U, Boston, MA

The cerebral cortex of the human brain contains a very extensive network of acetylcholinesterase (AChE)-rich neurons, located predominantly in layers III and V. Their density and laminar organization display marked variations which obey architectonic and functional boundaries. Although these neurons almost certainly represent at least one subset of cholinoceptive neurons, there is an inverse relationship between their density and that of intracortical cholinergic fibers. Cholinergic innervation is denser in limbic-paralimbic than in isocortical association areas. However, the density of AChE-rich intracortical neurons is higher in posterior parietal and prefrontal association areas than in limbic-paralimbic areas. In the visual and auditory systems, primary koniocortex has a lower number of these neurons than the adjacent unimodal association areas. In association neocortex, most of the AChE-rich neurons are located in LIII. In paralimbic areas, more of these neurons are found in the deeper cortical layers. Primary motor cortex is the only region with an equidense distribution in LIII and LV.

Our previous studies showed that the AChE-rich staining pattern of these neurons is absent at birth, starts to become established during late childhood (around 10 years of age), reaches a peak in the course of adulthood and does not show a consistent decline even in advanced senescence. The expression of high AChE levels in these neurons may indicate an increase in neuronal activity and plasticity in a way that may underlie the more advanced phases of human cognitive development.

434.17

IMMUNOSTAINING FOR MICROTUBULE-ASSOCIATED PROTEINS (MAP-1,-2,-5) IS DECREASED BY IBOTENIC ACID LESIONS OF THE CHOLINERGIC BASAL FOREBRAIN.

N.J. Woolf, J.D. Oh, and L.L. Butcher. Dept. of Psychology, UCLA, Los Angeles, CA 90024-1563.

In order to determine whether or not the basal forebrain cholinergic input has any effect on the presence of MAPs in the neocortex, we infused 10 µg of ibotenic acid in 1 µl saline into two sites of the rodent nucleus basalis (0.8 mm posterior, 2.5 mm ventral to bregma, and 7.0 mm ventral from the cortical surface; 1.8 mm posterior, 3.5 mm lateral to bregma, and 6.3 mm ventral to the cortical surface). Following survival times of 2 hr, 1 day, or 8 days, brains were processed for the immunohistochemical localization of MAP-1, MAP-2, and MAP-5 (antibodies purchased from Sigma Chemical Co., St. Louis, MO). Histochemistry for acetylcholinesterase (AChE) was also performed. Immunostaining for all three MAPs was markedly reduced in cortical somata and processes 8 days after lesion, but not at 2 hr or 1 day. Decreases were greatest for MAP-1, followed by MAP-5 and MAP-2, and all MAP proteins were decreased to the greatest extent in layer VI where basilar dendrites of pyramidal cells predominate. Decreases in MAP staining paralleled loss of AChE stained fibers in the cortex. MAP and AChE decreases were found in lateral neocortical fields but not in the piriform or cingulate cortices. These results suggest that cholinergic afferents may transynaptically regulate the expression of MAPs in cortical cells. [Support: USPHS grant NS 10928 to L.L.B.]

434.18

MAPPING OF CHOLINE ACETYL TRANSFERASE (ChAT) CELLS IN THE RAT BRAIN: POSSIBLE EVIDENCE FOR CHOLINERGIC INPUT TO THE CEREBELLUM. S.A. Azizi, A.J. Painchaud and D.J. Woodward, Dept. Cell Biology and Neuroscience, U.T. Southwestern Med. Sch., Dallas, TX 75235

The aim of this study was first to map the entire distribution of ChAT-containing neurons in the rat brain and, secondly, to determine whether ChAT-containing neurons in the brain stem project to the cerebellum.

ChAT-containing neurons were labelled using a polyclonal antibody to ChAT and conventional anatomical visualization techniques in 16 hooded rats. In 6 of these rats, HRP retrograde tracing techniques were also used to double-label the ChAT-containing neurons projecting to the cerebellum. Computer-assisted imaging techniques were utilized to analyze the distributions.

Our results confirmed previous reports on the distribution of ChAT labeled cells in the forebrain. Labelling was found in the medial septal nucleus, diagonal band of Broca, substantia innominota, corpus striatum, nucleus accumbens, lateral-globus pallidus and basal nucleus of Meynert. Within the brain stem, labeled neuronal groups included the pedunculopontine nucleus, the parabrachial nucleus and cranial motor and parasympathetic nuclei. In addition, several isolated clusters of labeled neurons previously unidentified were visualized within the pontine and medullary reticular formation. Furthermore, groups of weakly labeled cells were observed in the lateral reticular nucleus, around the trapezoid body and in locus coeruleus. Results from the double-labelling studies demonstrated that ChAT-containing lateral reticular neurons project to the cerebellum. *Supported by DA02338, AA03901 and the Biological Humanics Foundation.*

434.19

ACh SYNTHESIS IN DISSOCIATED PORCINE INTERMEDIATE LOBE CELLS. A. Tandon[1], B. Collier[1], Z.W. Zhang*, P. Feltz*
[1]Dept. of Pharmacology, McGill Univ., Montreal, Canada. *Inst. de Physiologie, Univ. Louis Pasteur, France.

Studies of the pituitary suggest a cholinergic component in the regulation of the intermediate lobe (IL). The origin of the cholinergic activity is unknown. In the anterior pituitary, choline acetyltransferase (ChAT) appears to be co-localized to cells that contain POMC. We tested whether the porcine IL, which is a homogeneous collection of POMC-containing cells, contains cholinergic markers. The corticotrophic tumor cell line AtT_{20} was used as a control since it can release acetylcholine (ACh). The ACh content (pmol/mg prot) increased from 209±15 in freshly dissociated IL cells, to 214±22 or 324±33 in 2 or 4-day IL cell cultures, respectively; AtT_{20} cells had 311±48. ChAT activity (nmol/mg prot/hr) was twice as high in the AtT_{20} cells (5.60±0.5) as compared to the freshly dissociated IL cells (2.87±0.6) and the 2 or 4-day cultures (1.95±0.2, 1.76±0.3). Napthylvinylpyridine (50uM), decreased ChAT activity in the freshly dissociated IL cells by 53%. AtT_{20} cells accumulated more [^{14}C]ACh (1544±240 dpm/mg prot) from exogenous [^{14}C]Ch (1uM) than the freshly dissociated IL cells (630±54 dpm/mg prot) or the 2 or 4-day cultured IL cells (763±64, 997±242 dpm/mg prot). Hemicholinium-3 (30uM), decreased the accumulation of [^{14}C]ACh in the IL cells. We conclude that IL cells can synthesize and store ACh.

434.20

SINGLE CHOLINERGIC MESOPONTINE TEGMENTAL NEURONS PROJECT TO BOTH THE PONTINE RETICULAR FORMATION AND THE THALAMUS IN THE RAT. Kazue Semba, Peter B. Reiner and Hans C. Fibiger. Div. of Neurol. Sci., Dept. of Psychiat., Univ. of British Columbia, Vancouver, B.C., V6T 1W5, and Dept. of Anat., Dalhousie Univ., Halifax, N.S. B3H 4H7 Canada.

Microinjections of carbachol into the pontine reticular formation (PRF) induce a REM sleep-like state. This carbachol-sensitive PRF region is innervated by cholinergic neurons in the pedunculopontine and laterodorsal tegmental nuclei. The same population of cholinergic neurons also projects to the thalamus, where there is good evidence that acetylcholine facilitates sensory transmission and blocks rhythmic thalamocortical activity. The present study was undertaken to examine the degree to which single cholinergic mesopontine neurons project to both the carbachol-sensitive PRF region and the thalamus, by combining double fluorescent retrograde tracing and immunofluorescence with an antibody to choline acetyltransferase. The results indicate that a subpopulation (5-21% ipsilaterally) of cholinergic mesopontine neurons projects to both the thalamus and the carbachol-sensitive site of the PRF. The percentage of cholinergic neurons with dual projections was higher in the pedunculopontine (6-27%) than in the laterodorsal (4-11%) tegmental nucleus. In addition, mixed with cholinergic neurons, there was a small population of dually projecting tegmental neurons that did not appear to be cholinergic. Mesopontine cholinergic neurons with dual projections may simultaneously modulate neuronal activity in the pontine reticular formation and the thalamus, and thereby have the potential of concurrently regulating different aspects of REM sleep.

434.21

PEPTIDE AND GLUTAMIC ACID DECARBOXYLASE mRNAs IN THE HUMAN NUCLEUS BASALIS OF MEYNERT. L.C. Walker, N.E. Rance*, D.L. Price and W.S. Young III. Neuropathology Laboratory, The Johns Hopkins University School of Medicine, Baltimore, MD 21205, and Laboratory of Cell Biology, NIMH, Bethesda, MD 20892.

Neurons with mRNAs coding for peptides and glutamic acid decarboxylase (GAD) in the anterior and intermediate nucleus basalis of Meynert (nbM) were studied in four humans using hybridization histochemistry with radiolabeled, 48-base oligodeoxynucleotide probes. GAD mRNA was found mostly in small and medium-sized neurons scattered among unlabeled, large neurons of the nbM. Substance P, somatostatin, neuropeptide Y, and enkephalin mRNAs were found in minor populations of mostly smaller neurons; these hybridoreactive neurons corresponded in size and distribution to immunoreactive peptidergic somata previously identified in the nbM of macaques. Galanin mRNA occurred only in a small number of human nbM neurons, in contrast to the extensive presence of galanin-hybridoreactive neurons in the nbM of nonhuman primates. Finally, neurokinin B mRNA was found in a subset of magnocellular neurons in humans. Thus, numerous noncholinergic markers occur in neurons of the human nbM, some of which are integral components of the nucleus and others of which may belong to separate but anatomically overlapping structures.

434.22

Autoradiographic Analysis of Pre- and Post-Synaptic Muscarinic Cholinergic Pathways in Bat, Shrew and Rat Brain. M.M. Howland, D.S. Higgins, R.L. Albin, K.A. Frey. Dept. of Neurology, Univ. of Michigan, Ann Arbor, MI 48109.

Studies in rodents and primates have characterized the CNS pathways subserving muscarinic cholinergic (mus/chol) neurotransmission. To assess the comparative anatomy of mus/chol pathways we used quantitative film autoradiography to measure pre- and post-synaptic markers of mus/chol function in three phylogenetically diverse groups of mammals, including shrews, microchiroptera, macrochiroptera, and rats. High affinity cholinergic uptake sites and cholinergic vesicles were measured with [^{3}H]hemicholinium and [^{3}H]vesamicol, respectively. Mus/chol receptors were measured with [^{3}H]scopolamine. The regional distribution of pre- and post-synaptic markers was similar in all subjects studied. Receptor density was highest in hippocampus, striatum, amygdala, and cerebral cortex, intermediate in thalamus and brainstem, and lowest in cerebellar cortex. Binding of pre-synaptic markers was highest in the interpeduncular nucleus and progressively lower in amygdala, striatum, hippocampus, neocortex, thalamus, and cerebellar cortex. These results indicate conservation of mus/chol pathways among eutherian mammals. Supported by NS01300, MH42652.

ACETYLCHOLINE—RECEPTORS: MUSCARINIC III

435.1

COMPARISON OF CHOLINERGIC ANTAGONISTS ON RECEPTOR BINDING, ACETYLCHOLINE LEVELS AND MEMORY IMPAIRMENT IN RATS. F. P. Bymaster and H. E. Shannon*. Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN 46285.

The purpose of the present studies was to determine if cholinergic antagonists inhibited M1 muscarinic receptor binding ex vivo, altered ACh levels and impaired memory in the same dose range. The muscarinic antagonists scopolamine (S), trihexyphenidyl (THP) and pirenzepine (PZ) and the nicotinic antagonist mecamylamine (M) inhibited the ex vivo binding of the M1 antagonist ^{3}H-PZ to cortical whole homogenates with ED_{50}s of 0.05, 0.1, 50 and >10 mg/kg s.c. and for ^{3}H-QNB binding in M2-rich brain stem of 0.2, 5, >100 and >10 mg/kg, respectively. These antagonists inhibited ^{3}H-PZ binding in vitro in cortex with IC_{50}s of 2, 4, 7 and >10000 nM, and ^{3}H-QNB binding in brain stem with IC_{50}s of 4, 40, 1130 and >10000 nM, respectively. Doses of S, THP, PZ and M required to lower ACh levels to one-half maximum were 0.1, 0.4, >100 and >10 mg/kg s.c. Doses of S, THP, PZ and M required to disrupt memory in a spatial alternation paradigm were 0.1, 1.0, 30 and >10 mg/kg s.c. These data demonstrate that S and THP enter the brain well. Further, they interact with M1 receptors and lower ACh levels at doses in the same range. S was non-selective for M1 and M2 receptors, whereas THP was relatively selective for M1 receptors. PZ and/or active metabolites required relatively high doses to enter the brain and did not appreciably alter ACh levels. M did not interact with M1 or M2 receptors or alter ACh levels.

435.2

A MODEL OF THE MUSCARINIC RECEPTOR AGONIST PHARMACOPHORE DERIVED FROM COMPUTER-ASSISTED MOLECULAR DESIGN AND ANALOG STRUCTURE ACTIVITY RELATIONSHIPS (SAR). David Garvey, Charles Hutchins*, John Chung*, Youe-Kong Shue * and Michael McKinney Neuroscience and Drug Design Research Divisions, Pharmaceutical Discovery, Abbott Labs, Abbott Park. IL 60064.

Enhancement of cortical cholinergic tone as an approach to the treatment of the symptoms of dementia associated with Alzheimer's Disease has led to the search for novel cholinomimetics capable of penetrating the CNS and positively stimulating those muscarinic receptors in the cerebral cortex coupled to phosphatidylinositol hydrolysis (PI). Utilizing the primary amino acid sequence of the human m1 receptor (Peralta et. al., *EMBO J.*, 6, 3923,1987), we have developed a thee dimensional model of the receptor agonist ligand binding domain which rationalizes the binding affinities and the *in vitro* intrinsic activities of a series of non-quarternary muscarinic ligands. This model assumes three key points of interaction between the ligand and specific amino acid side chain residues on the receptor protein. Postulated are two hydrogen-bonds with the ligand involving Ser-109 and Asn-410. These interactions are presumed to remain constant throughout the process of agonist recognition binding and subsequent activation of the receptor PI response. In addition, we hypothesize that there is an electrostatic interaction between the ligand and the receptor which changes for the more efficacious agonists from Asp-105, important for recognition binding, to Asp-71, a requirement for ligand mediated receptor activation. The SAR of a series of spirocyclic piperidine and more rigid spirocyclic quinuclidine derivatives support this model.

435.3

MUSCARINIC RECEPTOR SUBTYPE-SELECTIVITY OF PIRENZEPINE DERIVATIVES. Yishai Karton,* Jesse Baumgold,† Robert Paek,†* Barton Bradbury,* and Kenneth Jacobson, Laboratory of Chemistry, NIDDK, NIH, Bethesda, MD, and †Dept. of Radiology, George Washington University Med. Center, Washington, DC.

In an effort to identify structural features of the muscarinic antagonist pirenzepine (PZ) which confer selectivity for m_1-cholinergic receptors, we synthesized a number of derivatives of PZ having modifications of the *N*-methylpiperazine side chain. These derivatives were tested in binding assays vs. [^{3}H]*N*-methylscopolamine in membranes of transfected cells expressing a single receptor subtype. The potency/selectivity were highly dependent on substitutions of the *N*-methyl group. Replacement of the methyl group with H resulted in an m_1-selective compound which was one order of magnitude less potent than PZ. Replacement of this methyl with a 2-chloroethyl group resulted in a compound that displayed affinities similar to PZ at each of the m_1-m_4 muscarinic receptor subtypes. This compound proved to be an irreversible inhibitor of muscarinic receptors after cyclizing to the chemically reactive aziridinium ring and may become a useful tool with which to study the binding domain of each receptor subtype. Finally, a "functionalized congener" design approach was applied to the *N*-methyl group resulting in chemically functionalized *N*-alkyl analogs. For example, a non-selective derivative containing a 6-methylene spacer chain was approximately equipotent to PZ at m_1 receptors and considerably more potent than PZ at the other muscarinic subtypes.

435.4

New Antagonists of Central Muscarinic Activity: 1-Methyl-3-(5-alkoxy-4-alkyloxazol-2-yl)-1,2,5,6-tetrahydropyridines. C.H. Mitch, H.E. Shannon*, D.D. Schoepp, D.T. Wong, F.P. Bymaster*, S.J. Quimby*. Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN 46285.

A series of the title compounds have been found to be potent muscarinic antagonists by both *in-vitro* and *in-vivo* measures. Affinity for M_1 receptors was determined in binding studies using ^{3}H-pirenzepine in rat hippocampus. Binding studies in rat brain stem using ^{3}H-quinuclidinyl benzilate in the presence of unlabeled pirenzepine were used to determine affinity for M_2 receptors. Affinity for oxotremorine-M binding sites in rat hippocampal membranes was also determined. Antagonist activity was measured *in-vitro* through measurement of inhibition of acetylcholine stimulated phosphatidyl-inositol turnover in rat cerebral cortex slices. Reversal of oxotremorine-induced tremors in mice was used to measure antagonist activity *in-vivo*. Memory impairment was measured in a spatial alternation paradigm. The results for 1-methyl-3-(5-methoxy-3-(1-methylpropyl)-oxazol-2-yl)-1,2,5,6-tetrahydropyridine are representative of those found for the series: IC50's of 3 nM, 190 nM and 123 nM for displacement of ^{3}H-pirenzepine, ^{3}H-QNB, and ^{3}H-oxotremorine, respectively; IC50 of 69 nM for the inhibition of 100 uM acetylcholine stimulation of PI turnover; AD50 of 3 mg/kg for antagonism of oxotremorine-induced tremors in mice; ED50 of 1.0 mg/kg for impairing memory in rats.

435.5

THE M2 MUSCARINIC ANTAGONIST METHOCTRAMINE IS TOXIC TO HUMAN NEUROBLASTOMA CELLS SK-N-SH. N. Giladi, J.L. Cadet. Lab. of Preclinical Neurosciences, Columbia Univ., New York, NY 10032.

Cholinergic agonists that act at muscarinic receptors (mAChRs) have trophic influence on cell differentiation. Blocking of the mAChRs might cause cholinergic deprivation and deleterious effects on cellular function. In order to test that idea, we used the cholinergic human neuroblastoma cell line SK-N-SH to study the effects of specific antimuscarinic agents on cell survival in culture. The specific M2 mAChR antagonist methoctramine caused cell death in a dose response manner. 107 uM methoctramine caused 50% cell death after 24 hours. The specific M1 mAChR antagonist pirenzepine and the M3 antagonist 4-DAMP were without toxic effects. Preincubation with pertussis toxin (1ug/ml. for 24 hours) enhanced the toxicity of methoctramine but had no toxic effects on its own. These results demonstrate that blocking of the allosteric M2 mAChR site, which is present on the of SK-N-SH cells, can have lethal consequences. The present findings add further evidence for the trophic properties of acetylcholine and suggest a possible receptor-based approach to the treatment for patients with neuroblastoma tumors.

435.6

THE AMINOPYRIDAZINE MUSCARINIC AGONIST, SR 95639A, IS A FUNCTIONAL M_2 RECEPTOR ANTAGONIST IN RAT BRAIN. D.J. Anderson, E. Cadman*, L. Vella-Rountree*, S.P. Arneric and M. Williams. Neuroscience Research, Abbott Laboratories, Abbott Park, IL 60064-3500.

The minaprine analog, SR 95639A (morpholinoethylamino-3-benzocyclohepta-(5,6-c)pyridazine dihydrochloride) has been reported as an orally active, selective, and non-toxic muscarinic M_1 receptor agonist (Schumacher et al., Eur. J. Pharmacol. 166:139,1989).

Binding studies indicated that SR 95639A was moderately selective for the M_1 receptor irrespective of whether [^{3}H]pirenzepine binding was measured in cortex (IC_{50}= 1.88 ± 0.45 uM ;N=5) or hippocampus (IC_{50}= 1.01 ± 0.37 uM ;N=2). Activity at the brainstem M_2 receptor ([^{3}H]QNB) was similar (IC_{50}= 8.61 ± 2.40 uM ;N=3). The compound was inactive (> 100 uM; N=3) at displacing [^{3}H]MCC at the nicotinic cholinergic receptor.

Biochemical evaluation of the muscarinic activity of the compound showed it to be totally without effect on M_1 receptor-linked phosphatidyl inositol (PI) hydrolysis in cerebral cortex at concentrations up to 1.0 mM (0 % ;N=4), unlike carbachol and the oxadiazole arecoline analog, L 670,207 which had EC_{50} values of 116 ± 10 uM (N=6) and 0.83 ± 0.36 uM (N=3), respectively. Morever, SR95639A did not inhibit the M_1 response elicited by carbachol (7.4 ± 7.4 % at 100 uM ;N=3). SR 95639A also had no agonist activity at the M_2-receptor (0 % inhibition of forskolin-mediated activation of adenylate cyclase in striatum at 100 uM ;N=3). Similarly, SR 95639A did not alter basal levels of cGMP production, nor did it enhance (or inhibit) the cGMP production elicited by oxytocin in LLC-PK1 cells. The compound however antagonized the M_2-mediated inhibition of cAMP production by carbachol (10 uM) with a K_i of 5.3 ± 1.0 uM (N=5).

These findings suggest that: 1) SR 95639 is a functional M_2 receptor antagonist; and 2) the M_1 agonist properties exhibited in vivo cannot be correlated with actions at an M_1 receptor linked to PI hydrolysis.

435.7

Electrophysiological and Receptor Binding Characterisitics of Cholinergic Agents: Potential Therapeutics for Alzheimer's Disease. D.J. Critchett*, R.M. Mangano, E.J. Trybulski, B. Beer and D.E. Clody. Med.Res.Division, American Cyanamid Co. Pearl River, NY. 10965.

Alzheimer's disease (AD) is a neurodegenerative disease characterized by a profound memory loss among other cognitive dysfunctions and is the most common form of dementia. Many of the symptoms of AD correlate highly with a loss of cholinergic function. Our working hypothesis is that proper enhancement or restoration of cholinergic function may significantly reduce the severity of cognitive loss. We have targeted the cholinergic system as a therapeutic approach to alleviate some of the cognitive symptoms of AD. Ligands for muscarinic receptors were synthesized enantioselectively in order to probe the steric environment of the receptor and as a possible method to probe receptor subtype selectivity.

The purpose of this study was to confirm the *in vitro* biochemical predictions of efficacy by measuring the physiological responses to these compounds at the synaptic level. The results obtained confirm the intrinsic (agonist/antagonist) activity predicted by the receptor binding assays.

The receptor binding assays employed displacement of ^{3}H-QNB from rat cortical MAChR to identify compounds that specifically interact with this receptor and the determination of K_i values for the competitive displacement of ^{3}H-QNB (antagonist) and ^{3}H-*cis*methyldioxolane (agonist) to characterize the compounds' intrinsic physiological activity (full or partial agonist, antagonist). Intrinsic activity was studied by *in vivo* examination of the effects of novel compounds on hippocampal pyramidal cells extracellulary by microiontophoresis. Microiontophoretic application of muscarinic agonists stimulates pyramidal cell firing, an effect which is blocked by iontophoresis of cholinergic antagonists and possibly, by weak partial agonists. Subtype selectivity was assessed by the competitive displacement of ^{3}H-pirenzepine from rat cortical membranes and ^{3}H-QNB from rat cardiac membranes.

435.8

Affinity Labeling of Muscarinic Acetylcholine Receptors: Covalent Modification of the Ligand Recognition Site. E. J. Arnold, R. M. Mangano, D. J. Critchett*, H. Brabander* and E.J. Trybulski*. American Cyanamid Company, Medical Research Division, Pearl River, NY 10965.

Muscarinic acetylcholine receptors (MAChRs) belong to a large class of peripheral and central nervous system receptors which couple to guanine nucleotide binding proteins during signal transduction. Five genes have been cloned from rat and human tissue that encode MAChRs. The coding regions of these genes have provided the biochemist with the primary amino acid sequences of these receptors and have suggested a tertiary structure of seven trans-membrane domains homologous to the proposed structure of bacteriorhodopsin. Neurotransmitters for this class of receptors are cations whose energy of interaction with the receptor is derived from ion-pairing with an anionic residue. Aspartic acid residues in two of the transmembrane helices are conserved throughout this receptor class and are likely candidates to fulfill this function. This information has been very useful in the design of experiments to elucidate how agonists and antagonists interact with these receptors as well as which features of these receptors can be targeted by synthetic ligands. Data from such experiments can be very useful in the design of specific drugs targeted to MAChRs.

The studies presented here describe the molecular pharmacology of novel oxotremorine (muscarinic agonist) derivatives. The compounds were synthesized optically pure to provide additional information about the stereochemical requirements of ligand binding to MAChRs. The results indicate that these compounds bind non-reversibly to MACHRs and that this reaction can be blocked by oxotremorine. This covalent attachment is time-dependent and apparently enantioselective. Intrinsic activity was assessed by *in vivo* recording from hippocampal pyramidal cells after microiontophoretic application of the compounds. The results of these experiments and the potential use of these compounds will be presented.

435.9

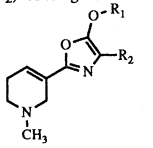
A NEW CLASS OF CHOLINERGIC ANTAGONISTS: ACRIDINE ARAPHANES HAVE A HIGH AFFINITY TO MUSCARINIC RECEPTORS.
K.P. Shaw[1], F.C.A. Silveira[1*], R.S. Aronstam[2] and E.X. Albuquerque[1,3]. [1]Dept. Pharmacol. Exp. Ther., Univ. Maryland Sch. Med., Baltimore, MD 21201, [2]Dept. Pharmacol. & Toxicol., Med. Coll. Georgia, Augusta, GA 30912 and [3]Lab. Mol. Pharmacol. II, IBCCF, UFRJ, Rio de Janeiro 29141, Brazil.

Acridine araphane analogs (Himel et al., Science, 205:1277, 1979) are structurally similar to 9-amino-1,2,3,4-tetrahydroacridine (THA), which has been shown to improve the prognosis of patients with Alzheimer's disease. The objective of this study is to define the antagonistic efficacy of acridine analogs at nicotinic as well as muscarinic receptors. LD_{50}s in mice (i.p.) for 1,2 propane (1,2-PAA) 1,4-butane (1,4-BAA), 1,5-pentane (1,5-PeAA), 1,6-hexane acridine araphane (1,6-HAA), and THA were 87, 110, 70, 95 and 32 mg/kg, respectively. On muscarinic and nicotinic receptors, 1,2-PAA, 1,3-propane araphane acridine (1,3-PrAA), 1,4-BAA, 1,5-PeAA and 1,6-HAA inhibited the [^{3}H]-N-methyl-scopolamine (rat hippocampus, cortex, brain stem and atrium) and [^{125}I]α-BGT (Torpedo electric organ) binding to the receptors with IC_{50}s of 1-10 nM and 1-10 μM, respectively. pA_2 values of antagonist affinity from Schild plot for 1,4-BAA, 1,2-PAA in ileum-muscarinic receptor and atrium-muscarinic receptor were 9.2, 8.7 and 6.6, 6.9 respectively. These analogs (5-50 μM) reversibly blocked sciatic-sartorius indirect muscle twitch of frog. On endplate currents (EPCs) of frog sartorius muscles, 1,2-PAA, 1,3-PrAA, 1,4-BAA and 1,6-HAA displayed a voltage-dependent decrease the decay time constant and depression of the peak amplitude in a concentration (0.5-5 μM)-dependent manner. THA blocked the EPC decay and peak amplitude at 50 μM. 1,2-PAA, 1,4-BAA and 1,6-HAA enhanced desensitization in denervated rat muscles, but THA depressed the amplitude of acetylcholine transients. In summary, results from biochemical and biological assays demonstrated that the acridine araphanes have higher affinity than THA for cholinergic receptors. Furthermore, acridine araphanes have a higher affinity (100-1000 fold) for muscarinic receptors than for nicotinic receptor.
(Support: US Army Med. Res. & Devel. Comm. Contract DAMD 17-88-C-8119).

435.10

OXAZOLE DERIVATIVES OF ARECOLINE WITH HIGH AFFINITY FOR MUSCARINIC RECEPTORS IN RAT BRAIN.
W.S. Messer, Jr. C.H. Mitch, B.R. Ellerbrock*, F.P. Bymaster*, H.E. Shannon* and W. Hoss. Medicinal & Biological Chemistry, College of Pharmacy, Univ. of Toledo, 2801 W. Bancroft St., Toledo, OH 43606; Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN 46285.

A series of alkoxy- and alkyl-oxazole derivatives of arecoline was synthesized for evaluation as novel muscarinic ligands. Substitution of the oxazole ring with either methoxy or propoxy moieties (R_1) and either 1-methylpropyl or 2-methylpropyl functions (R_2) led to ligands with markedly different binding properties.



LY 191492: R_1=propyl; R_2=1-methylpropyl

LY 209127: R_1=methyl; R_2=1-methylpropyl

LY 191309: R_1=propyl; R_2=2-methylpropyl

LY 242168: R_1=methyl; R_2=2-methylpropyl

Binding was examined through quantitative autoradiographic techniques in rat brain. The ability of each ligand to displace [^{3}H]-*l*-quinuclidinyl benzilate (QNB) binding to brain sections was compared with the known distribution of M_1 and M_2 muscarinic receptors as measured previously with pirenzepine and AF-DX 116. LY 191492 displayed the highest affinity for [^{3}H]-*l*-QNB binding sites, while LY 209127, LY 191309 and LY 242168 had lower affinities for muscarinic receptors.

Quantification of the autoradiograms indicated that LY 191492 bound with the highest affinity to M_1 receptors in the dentate gyrus (IC_{50} = 2.0 nM), and had a lower affinity for thalamic receptors (IC_{50} =19 nM). LY 209127 displayed a lower affinity for muscarinic receptors with IC_{50} values near 50 nM and a relative lack of selectivity. The binding of LY 191309 and LY 242168 was of even lower affinity especially for M_2 muscarinic receptors in the superior colliculus. The data indicate that the oxazole derivatives have a wide range of activities and different selectivities within the CNS.
Supported by NS 23929, NS 25765 and Eli Lilly and Company.

435.11

CHARACTERIZATION AND AUTORADIOGRAPHIC DISTRIBUTION OF [^{3}H]AF-DX 384 MUSCARINIC BINDING SITES IN THE RAT BRAIN.
S.Gauthier, D.Cécyre*, I.Aubert and R.Quirion. Douglas Hospital Research Centre, McGill University, Dept. Neurology & Neurosurgery and McGill Centre for Studies in Aging, Montreal, Quebec, Canada H4H 1R3.

Highly selective and specific radioligands are available for the study of muscarinic M1 receptor sites (e.g. pirenzepine). However, such probes are mostly lacking for the investigation of the M2 receptor subtype, although [^{3}H]acetylcholine, [^{3}H]oxotremorine-M, [^{3}H]*N*-methylscopolamine and [^{3}H]AF-DX 116 have been used in the past. We present here the characterization of [^{3}H]AF-DX 384 as a new selective M2 ligand.

[^{3}H]AF-DX 384 binds to saturable population of sites in rat forebrain membrane preparation with 75% to 80% of total binding being specifically bound at 2nM. The ligand selectivity profile reveals that (-)QNB (IC_{50}=0.5nM) is more potent than > atropine (0.6nM) > AQ-RA 741 (4.1nM) > AF-DX 384 (7.6nM) > UH-AH 371 (14.9nM) >> oxotremorine-M (81nM) > pirenzepine (220nM) >>> nicotine (>10uM). This suggests that [^{3}H]AF-DX 384 mostly binds to a M2-like population of muscarinic sites in the rat central nervous system.

This is further supported by an autoradiographic study which reveals a M2-like distribution pattern, with high labelling seen in areas such as the superior colliculus, pontine nucleus, motor trigeminal nucleus, certain thalamic nuclei and to some extent, in the cerebellum. Similar localization have been previously reported using other M2 receptor probes. However, autoradiograms are of better qualities with [^{3}H]AF-DX 384 because of the higher specificity and stability of this ligand. Thus, [^{3}H]AF-DX 384 should become a most useful probe for further characterization of brain M2 receptor sites. (MRC, Canada).

435.12

CHARACTERIZATION AND LOCALIZATION OF M_2 MUSCARINIC RECEPTORS LABELED WITH [^{3}H]AFDX384.
M. Hunt, M.E. Alburges, U. Busch[1], C.A. Belohlavek and J.K. Wamsley. Neuropsychiatric Res. Inst., Fargo, ND 58103; [1]Biochemistry Department, Thomae GmbH, Biberach FRG.

Thomae Company has introduced a new compound selective for the M_2 muscarinic cholinergic receptor subtype. This compound has been used to characterize M_2 receptor binding in tissue slices and to localize this receptor subtype by autoradiography. [^{3}H]AFDX384 was shown to label M_2 receptors in a selective manner and the binding was readily reversible and highly specific (85%). Saturation isotherms indicated a multiphasic binding pattern and conditions were restricted to obtain selective labeling to the high affinity sites which showed a K_d in the low nanomolar range.

Autoradiographic localization of M_2 sites with [^{3}H]AFDX384 indicate a predominance of these sites in regions of the brain formerly shown to contain significant concentrations of M_2 receptors. The order of binding was caudate-putamen = nucleus accumbens = superior colliculus > thalamus > nucleus of the solitary tract > cortex. [^{3}H]AFDX384 appears to be a viable ligand for use, under appropriate conditions, for obtaining specific labeling to M_2 receptors in the brain. The increased sensitivity and better stability make [^{3}H]AFDX384 superior to its predecessor, [^{3}H]AFDX116.

435.13

A NOVEL SIGNAL TRANSDUCTION MECHANISM FOR BRAIN MUSCARINIC RECEPTORS: STIMULATION OF ADENYLATE CYCLASE ACTIVITY.
P. Onali and M.C. Olianas. Department of Neurosciences, University of Cagliari, Italy.

In the central nervous system activation of muscarinic receptors has generally been shown to produce a decrease of cyclic AMP formation. We now report that in rat olfactory bulb homogenate carbachol (CCh) and other cholinergic agonists stimulate adenylate cyclase (a.c.) activity. The CCh stimulation shows a rapid onset, is readily reverted by atropine and is Ca^{2+}-independent, as it occurs in membranes prepared in the presence of 1 mM EGTA and incubated in a Ca^{2+}-free assay medium. Without added GTP, CCh fails to affect a.c.. With 1 mM CCh, the maximal stimulation is observed at 100 µM GTP (49 % increase of basal activity). The CCh response is not affected by staurosporine (0.01-1.0 µM), indomethacin (1-100 µM) and nordihydroguaiaretic acid (1-100 µM). Moreover, the muscarinic effect is additive with the enzyme stimulation elicited by phorbol 12-myristate 13-acetate (5 µM), is reduced by pertussis toxin (PTX) but not by cholera toxin.

These results indicate that in rat olfactory bulb muscarinic receptors are positively coupled to a.c. through a Ca^{2+}-independent and PTX-sensitive mechanism.

435.14

PHARMACOLOGICAL CHARACTERIZATION OF MUSCARINIC RECEPTORS MEDIATING ACETYLCHOLINE STIMULATION OF ADENYLATE CYCLASE IN RAT OLFACTORY BULB.
M.C. Olianas and P. Onali, Dept. of Neurosciences, University of Cagliari, Italy.

We have recently reported that in rat olfactory bulb acetylcholine (ACh) stimulates adenylate cyclase (a.c.) activity by acting on muscarinic receptors (Naunyn-Schmied Arch Pharmacol 1990). In the present study, we have investigated the pharmacological properties of the muscarinic receptors mediating the stimulation of a.c.. These receptors were activated by ACh in a concentration-dependent manner (EC 50 = 0.3 µM) with a maximal increase reached at 100 µM ACh. Different cholinergic agonists mimicked the effect of ACh with the following rank order of potency : oxotremorine ≥ oxotremorine M > ACh > carbachol=methacholine > bethanechol. As compared to ACh, the relative efficacies of oxotremorine and bethanechol were equal to 75% and 40%, respectively. Different muscarinic receptor antagonists competitively counteracted the ACh effect with the following ki values: atropine 0.6 nM, 4-DAMP 5 nM, AF-DX 116 130 nM, pirenzepine 310 nM. These results indicate that the muscarinic receptors mediating stimulation of a.c. are pharmacologically different from M_1 and cardiac M_2 and similar to M_3 receptor subtypes.

435.15

EVIDENCE FOR THE PRESENCE OF A NONCLASSICAL M4 PHARMACOLOGICAL MUSCARINIC RECEPTOR SUBTYPE (mAChR) IN RAT BRAIN REGIONS DETECTED BY A NOVEL MUSCARINIC ANTAGONIST DAU 6202. H. Ladinsky and G.B. Schiavi*. Department of Biochemistry and Molecular Pharmacology, Istituto De Angeli, Boehringer Ingelheim Italia, Milano 20139 Italy.

Inhibition of (^{3}H)PZ binding to cortical M1 mAChRs and (^{3}H)NMS binding to cardiac M2, glandular M3 and brain regional mAChRs was studied with a new antimuscarinic, DAU 6202 (4-hydroxy-3-(tropyl) oxycarbonyl-3,4-dhydro-1 H-quinazoline-2-one). DAU 6202 bound to the mAChRs with K_Ds of (nM): 1.8, M1; 4.3, M3; 204, M2. In striatum, a shallow inhibition curve was seen showing that DAU 6202 bound to a heterogeneous population of sites. Computer analysis showed that DAU 6202 bound 31% of total sites with a K_D of 3 nM and 69% with a K_D of 42 nM. The 3 nM site likely represents M1 and M3 mAChRs detected by blot hybridization (Buckley et al., J. Neurosci. 8, 4646, 1988). The 42 nM site, non M1, non M2, non M3 (termed M4), may be the m4 mAChR recognized by hybridization. In other brain regions, DAU 6202 gave shallow inhibition curves revealing M4 sites of major proportions, i.e. cortex, 38%; hippocampus, 33%; olfactory bulb, 46%; hypothalamus, 56% of total sites.

RESPIRATORY CONTROL

436.1

NEURONS IN THE VENTRAL PART OF THE NUCLEUS TRACTUS SOLITARIUS EXHIBIT MULTIPLE TYPES OF CALCIUM CURRENTS. M.S. Dekin, Comparative Neurobiology and Physiology Group, School of Biological Sciences, University of Kentucky, Lexington, KY 40506-0225.

Premotor respiratory neurons in the guinea pig are located in the ventral parts of the nucleus tractus solitarius (vNTS). Using brainstem slices from adult guinea pigs, the properties of calcium currents in identified bulbospinal neurons in the vNTS were studied. Two types of calcium currents could be distinguished using the single electrode voltage clamp technique; a low voltage-activated (LVA) current which was expressed during depolarizations between -50 and -20 mV and a high voltage-activated (HVA) current which was expressed during depolarizations more positive than -35 mV. Both the LVA and HVA currents were blocked by the addition of 1 mM Cd^{++} to the bath. During the course of a 500 msec step depolarization to -30 mV from a holding potential of -50 mV, the LVA current exhibited almost complete inactivation. Partial inactivation of the HVA current was also observed but was much slower in onset. During similar depolarizations from holding potentials more positive than -50 mV only the HVA current was observed suggesting that the LVA current underwent steady-state inactivation. Under these conditions, the LVA current could only be observed when depolarization was preceded by a hyperpolarizing voltage step to levels more negative than -50 mV. These data suggest that expression of the LVA calcium current during inspiratory phase depolarization in premotor respiratory neurons would be dependent upon the level of membrane hyperpolarization achieved during previous expiratory phase inhibition. (Supported by NIH grants HL40369 and HL02314, and University of Kentucky BRSG Grant RR07114).

436.2

EFFECT OF HISTOTOXIC ANOXIA ON HYPOGLOSSAL NEURONS STUDIED BY WHOLE-CELL PATCH-CLAMP. T.R. Cummins*, D.F. Donnelly and G.G. Haddad, Dept. of Pediatrics (Div. Respiratory Medicine), Yale Univ., New Haven, CT 06510.

During anoxia, brainstem neurons in the slice preparation show an increase in excitability while hippocampal neurons show a decrease. In this work, we study the membrane properties of acutely isolated Hypoglossal [XII] neurons during anoxia and compare them to Hippocampal CA1 cells using the whole-cell patch-clamp technique. Neurons dissociated from XII of 7 and 26 day old rats exhibited resting potentials in the range of -43 to -70 mV and action potentials of 75 to 105 mV in the current clamp mode. These neurons fired repetitively with little adaptation and no delayed excitation (A-current). Cyanide (CN) depolarized XII neurons and caused an increase in excitability (n=8). When recording in the voltage-clamp mode, CN (4mM) caused an increase in the voltage-dependent outward current (121±5% of control, mean + SEM, n=13) and in 7 out of 8 cells CN increased the voltage-dependent inward current (stepping from -100 mV to -10 mV). These results show that 1) acutely isolated Hypoglossal neurons can be studied using whole-cell patch-clamp, 2) in contrast to Hippocampal CA1 neurons (Fed. Proc. 4:A405, 1990), CN causes an increase in the voltage-dependent inward and outward currents of XII neurons and 3) differences between brain regions in neuronal response to hypoxia may be due to intrinsic neuronal properties.

436.3

CHEMOSENSITIVITY OF MEDULLARY AND HYPOTHALAMIC NEURONS IN CELL CULTURE. W. Chou, J.A. Neubauer, H.M. Geller and N.H. Edelman.* UMDNJ-Robert Wood Johnson Medical School, New Brunswick, N.J. 08903.

To determine whether dissociated neurons in vitro retain the function of CO_2 chemosensitivity and whether this is specific to the ventral medulla, CO_2 sensitivity was characterized in neurons (1-4 weeks in vitro) using loose-cell patch recordings (Rs 77±10 MΩ). Dissociated cell cultures were prepared by enzymatic and mechanical treatment of ventral (VM) and dorsal (DM) medulla of neonatal rats and medulla (MED) and hypothalamus (HYPTH) of E17 rats. Spontaneously active neurons were found in all brain regions: VM 8/45, DM 2/12, MED 27/56 and HYPTH 40/114. Although spontaneous activity increased (5% to 40%) from 1 to 4 weeks in vitro, firing frequency (range <1-4 Hz) was unchanged. Superfusate pH was varied by altering PCO_2 (14-71 Torr). While 55% of neurons were unresponsive to changes in pH, increases (20%) and decreases (25%) in activity with acidosis were observed in all regions. We conclude that: (1) neurons in dissociated cell culture retain the function of CO_2 chemosensitivity; (2) CO_2 chemosensitivity is present in both medullary and hypothalamic cell cultures; and (3) as previously shown, the number of spontaneously active neurons increases with time in culture due to the development of functional synaptic connections. Supported by HL33938, HL16022 and NS24168.

436.4

SPINAL EFFECTS OF TACHYKININ PEPTIDES ON RESPIRATORY PUMPING MUSCLES. M.A. Haxhiu*, B. Erokwu*, E. van Lunteren, and N.S. Cherniack. Departments of Medicine and Neurosciences, Case Western Reserve University, Cleveland, OH.

The tachykinin peptides and their receptors [e.g. substance P (SP) and neurokinin A (NKA)] are present in the spinal cord, including the ventral horn. Their role in the regulation of respiratory pumping muscle activity at the spinal level is not well understood. In anesthetized spontaneously breathing cats we examined the effects of SP and NKA administered into the spinal intrathecal space at C5-C7 on the electromyographic activity of the diaphragm (D_{EMG}), inspiratory parasternal intercostal (Ii_{EMG}) and expiratory triangularis sterni (Ei_{EMG}) muscles. SP in doses of 10-100 nmol increased the electrical activity of the respiratory muscles in 8 of 10 cats, causing D_{EMG} to change from 21 ± 2 to 29 ± 4 units ($p < 0.05$), producing an increase of Ii_{EMG} from 16 ± 3 to 27 ± 4 units ($p < 0.05$), and elevating Ei_{EMG} from 12 ± 2 to 25 ± 3 units ($p < 0.05$). Similar stimulatory effects occurred following NKA given to 2 cats. Tachykinins administered to the spinal cord caused an insignificant change in inspiratory and expiratory timing, and induced a slight but insignificant increase in arterial blood pressure (16 ± 8%) and heart rate (2 ± 1%). The results suggest that mammalian tachykinins might be involved in the regulation of respiratory muscle activity by acting directly on motoneurons or interneurons at a spinal level.
Support: NIH HL-38701 and HL-01600.

436.5

GLUTAMATE AND GABA CONTENT OF MICRODIALYSATES FROM CAT PHRENIC NUCLEUS. C.A. Connelly, L.L. Sommers*, & J.L. Feldman. Systems Neurobiology Laboratory, Dept. of Kinesiology, UCLA, Los Angeles, CA 90024-1568.

In vivo microdialysis was used to directly assay the glutamate (GLU) and GABA content in the phrenic nucleus (PN) in chloralose-urethane anesthetized, vagotomized, artificially ventilated cats. The PN in the C5 segment of the spinal cord was localized by mapping with a saline-filled extracellular microelectrode. The recording electrode was then removed and the microdialysis probe (1 mm length x 500 μm diameter dialysis membrane) inserted at the location where spontaneous inspiratory-modulated discharge was recorded and antidromic spikes were elicited by stimulation of the phrenic nerve. The probe was perfused with Ringer's solution (pH=7.5) at a rate of 1 μl/min for collection (every 20 minutes) of samples (20 μl) containing dialyzed amino acids from the extracellular fluid in the PN. Amino acid content of each sample was determined by high performance liquid chromatography with electrochemical detection (BAS 200). Mechanical insertion of the probe into the PN initially caused enhanced, non-specific amino acid release, with the initial dialysates containing GLU levels of 27 pmoles/10 μl and GABA levels of 1.4 pmoles/10 μl. After stabilization of the probe *in situ* for 1-3 hours, levels of GLU in the PN dialysates decreased to steady-state levels of 3 to 6 pmoles/10 μl, and remained constant for 4-8 hours. GABA levels in the dialysates ranged from 0.5-1.0 pmoles/10 μl after reaching the steady-state. The concentration of amino acid in the PN was estimated to be 10 times that of the dialysates. This estimate is based on a 10% efficiency of dialysis probe recovery of amino acid content in a sampled region, as calculated from *in vitro* testing of probe recovery characteristics in known concentrations of amino acid standards. After each experiment, the probe locations were verified histologically and found to be centered in the PN. The extracellular content of GLU and GABA in the PN is consistent with the hypotheses that GLU and GABA mediate bulbospinal excitatory (inspiratory) and inhibitory (expiratory) inputs on phrenic motoneurons, respectively. Research supported by NIH grant HL 37941. CAC is a Parker B. Francis Foundation Fellow.

436.6

MULTIPLE NEUROMESSENGERS ARE CONTAINED IN TERMINAL VARICOSITIES IN RAT PHRENIC NUCLEUS. H.H. Ellenberger[1], P.L. Vera[2], J.L. Feldman[1] & V.R. Holets[2]. [1]Systems Neurobiology Lab., Dept. of Kinesiology, UCLA, L.A., CA 90024-1568 and [2]Univ. of Miami Medical School, Dept. of Neurological Surgery, Miami, FL 33136.

Combined horseradish peroxidase labeling of phrenic motoneurons and immunohistochemical detection of 9 putative neuromessengers (5-hydroxytryptamine [5-HT], substance P [Sub. P], thyrotropin-releasing hormone [TRH], methionine enkephalin [M. Enk], neuropeptide Y [NPY], polypeptide YY [PYY], cholesystokinin [CCK], somatostatin [SST] and neurotensin [NT]) revealed that a wide variety of neuromessengers are contained within terminal varicosities in the phrenic nucleus. The rank order for the relative degree of terminal labeling (based on visual inspection) in the phrenic nucleus was:

5-HT≈Sub. P>CCK>M. Enk≈TRH>NPY≈PYY≈SST>NT

The indirect immunofluorescence technique was utilized to demonstrate colocalization of 5-HT with either Sub. P, TRH or M. Enk within terminal varicosities in the phrenic nucleus. The coincidence of double-labeling varied for each peptide colocalized with 5-HT. Almost all varicosities labeled for 5-HT were also labeled for Sub. P. Some Sub. P labeled varicosities were not labeled for 5-HT, indicating a partial coexistence of SP with 5-HT. All TRH positive terminals were labeled for 5-HT, but there were some 5-HT labeled terminals not labeled for TRH. Varicosities labeled for both 5-HT and M. Enk. showed the lowest level of double-labeling, with many varicosities labeled for only 5-HT or M. Enk. These results indicate that phrenic motoneurons are subject to many sources of modulation that can alter their responsiveness to primary excitatory and inhibitory inputs. The ubiquitous presence of peptides within 5-HT neurons and possible corelease of these peptides with serotonin must be accounted for in studies of the role of the raphe nuclei in the control of breathing. Supported by NIH grants NS 24742 and HL 37941.

436.7

DIFFERENTIAL EFFECTS OF ANESTHESIA ON THE CO2 RESPONSES OF EXPIRATORY BULBOSPINAL (EBS) NEURONS AND PHRENIC NERVE ACTIVITY OF VAGOTOMIZED DOGS. E. Stuth, M. Tonkovic-Capin, E. Zuperku, and J. Kampine*Medical College of Wisconsin and VA Medical Center, Milwaukee, Wi 53295.

Data regarding the effects of various anesthetic regimens on central inspiratory (I) and expiratory (E) neural activities in form of CO2 response curves were obtained. Intravenous sodium thiopental (STP) monoanesthesia was compared with isoflurane (ISO) monoanesthesia at depths ranging from 0.5 to 2.5 minimum alveolar concentrations (MAC) as well as with combinations of the two anesthetics (STP + ISO). Hyperventilation with O2 and the addition of CO2-O2 mixtures were used to produce 3 levels of central CO2-drive, low(L), medium(M), and high(H) for each anesthetic depth. Plots of peak EBS neuron discharge frequency (Fn) vs increasing depth of anesthesia showed a progressive decrease in Fn for H and M drive levels. At L drive most EBS neurons fired tonically at all levels of anesthesia with little change in Fn. Peak phrenic activity (PPA) showed a more pronounced decline with increasing anesthetic depth at all levels of drive. Phrenic activity ceased at 2-2.5 MAC ISO monoanesthesia and at 1 MAC STP + ISO combination anesthesia even at H CO2 drive, while EBS neurons continued to fire tonically at 20-60 % of Fn at 1 MAC-H or STP-H levels of the monoanesthetic regimens. Our studies suggest that (1) I neurons are more sensitive to increasing depth of anesthesia than are E neurons, and (2) combinations of intravenous STP basal anesthesia and ISO inhalational anesthesia cause more pronounced decreases in central I activity than the respective monoanesthetic regimens.

436.8

UNILATERAL VENTROLATERAL MEDULLA (VLM) ELECTROLYTIC LESIONS RESULT IN APNEA OR DECREASED PHRENIC ACTIVITY AND DECREASED CO_2 SENSITIVITY IN THE ANESTHETIZED CAT. Eugene E. Nattie, Aihua Li* and Walter M. St. John*. Dartmouth Medical School, Hanover, N.H., 03756 and Jinling Hospital, Nanjing, P. R. C.

In previous studies, 10 nL injections of the neurotoxin, kainic acid, or the excitatory neurotransmitter, glutamate, in a region of the VLM ventral and ventromedial to the facial nucleus extending caudally to the level of the rostral aspect of the inferior olive resulted in phrenic apnea or decreased phrenic activity and decreased CO_2 sensitivity. To ascertain why both a destructive toxin and an endogenous excitatory amino acid should have the same effects, we produced unilateral lesions in decerebrate or chloralose urethane anesthetized, vagotomized, glomectomized, paralyzed, and servo-ventilated cats. Large radiofrequency lesions (70°C; 1 min) which included this region resulted in apnea. Smaller electrolytic lesions (5-10 mA; 15 sec) which included, and in some cases were limited to, this region resulted in apnea or decreased phrenic activity and decreased CO_2 sensitivity. Stimulation prior to lesioning increased phrenic activity. We conclude that kainic acid injections and lesions destroyed cells in this region which appear to be a source of tonic excitatory input for ventilatory activity. We interpret the inhibitory effects of glutamate injections as due to depolarization blockade. (Supported by HL 28066).

436.9

PARABRACHIAL NEURON DISCHARGE IS ALTERED DURING THE CARBACHOL INDUCED REM SLEEP-LIKE STATE (DCARB). K.A. Gilbert and R. Lydic. Department of Anesthesia, Pennsylvania State University, College of Medicine, Hershey, PA, 17033.

The parabrachial nuclei are known to play a role in cardio-respiratory control. Some parabrachial neurons reveal altered discharge during rapid eye movement (REM) sleep but the mechanisms mediating these state-dependent firing rates remain poorly understood. Encouraged by the finding that microinjections of carbachol into the medial pontine reticular formation (mPRF) of intact, unanesthetized cats produce state-dependent respiratory changes paralleling those observed during REM sleep (Lydic & Baghdoyan, *Neurosci. Letts. 102*:211,1989), this study is testing the hypothesis that cholinergic mechanisms in the mPRF can also cause state-dependent changes in the discharge of parabrachial neurons. To date, 9 cells have been recorded with extracellular electrodes aimed for the parabrachial region in 4 cats. Cells were recorded across 16 cycles of waking (W), NREM, and REM sleep, and in 9 DCarb episodes. Qualitatively, cells included tonic and phasic respiratory and non-respiratory modulated neurons. Relative to W, 7 cells increased firing (mean % change) during both REM (40.6%) and Dcarb (36.1%) and 2 cells decreased in REM (34.3%) and Dcarb (47.9%). The 7 REM-on cells revealed firing rates in Dcarb which were not significantly different from firing rates in REM. These results are consistent with the hypothesis that cholinergic mechanisms in the mPRF can cause state-dependent changes in the discharge of parabrachial neurons.

Supported by grant HL-40881 to RL.

436.10

MEDULLARY SINGLE UNIT RESPONSES TO SYSTEMIC HYPOXIA (HYP) IN CHEMODENERVATED CATS. J. Mitra, N.B. Dev*, J.R. Romaniuk*, R. Trivedi*, and N.S. Cherniack. Department of Medicine, Case Western Reserve University, Cleveland, OH 44106.

We studied the effects of HYP (6% O_2) on 45 single medullary units located within 2mm of the ventral surface (P 8.0-P 12.0) in peripherally chemodenervated cats while recording phrenic and sympathetic activity.

Twenty seven percent (12 units) increased their activity during HYP. All of these units discharged tonically during normoxia. Three units discharged continuously throughout HYP but none could be antidromically (AD) activated from the intermediolateral cell column (IML). However, two of these units could be excited by proprioceptive stimuli. Five units had a biphasic response with initial excitation followed by depression and then by reappearance of excitation. A portion of these units could be stimulated AD but not by proprioceptive input. Units excited by HYP could be found both rostrally and caudally. No units with respiratory modulation (4 tested) increased by hypoxia.

Our results suggest that there are tonically discharging units in the ventral medulla that can be excited by hypoxia. Only a portion of these seem to project to the IML.

Support: NIH HL-25830.

436.11

RESPIRATORY PATTERNING FOLLOWING COCAINE ADMINISTRATION TO THE CENTRAL NUCLEUS OF THE AMYGDALA. R.K. Harper, R.R. Terreberry, R.C. Frysinger, C.A. Richard and R.M. Harper. Brain Research Institute and Department of Anatomy & Cell Biology, UCLA, Los Angeles, CA 90024.

We examined patterning of laryngeal dilator and diaphragmatic activity in intact, freely moving cats following administration of 3 dose levels of cocaine (0.625, 1.25, 2.5 mg) to the central nucleus of the amygdala (ACE). Diaphragmatic and laryngeal dilator EMG, EEG, nuchal EMG, EOG, hippocampal slow wave, and brain and core temperature recordings were continuously gathered before and following cocaine administration through an indwelling brain cannula. Both low- and high-dose cocaine administration was followed by tachypnea; panting intermixed with tachypnea also accompanied high-dose injection. Direct administration of cocaine to the ACE thus resulted in respiratory patterns similar to those observed following intravenous and intraventricular administration described earlier. We suggest that the ACE projections to phase-switching areas in the pons or the projections to the nucleus of the solitary tract may mediate a portion of the respiratory pattern changes following cocaine administration.

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436.12

MU VERSUS DELTA OPIOID RECEPTORS IN THE MODULATION OF RESPIRATORY PATTERN AND CONTROL IN EARLY POSTNATAL LIFE. S.C. Scott , J.D.G. Inman and I.R. Moss. Depts. of Pediatrics and Physiology, UT Southwestern, Dallas, TX 75235.

Endogenous opioid systems participate in the modulation of respiratory pattern and control in perinatal life. In an effort to determine which opioid receptor is responsible for this modulation, naltrexone (mu>delta>kappa antagonist) or naltrindole (delta antagonist) was administered intravenously to chronically instrumented, unanesthetized piglets. In 6-10 day old piglets, 1-2 mg/kg of naltrexone increased breathing frequency, the amplitude and rate of rise of diaphragmatic electromyographic activity (EMGdi) and the amplitude, rate of rise, total area and initial area of the posterior cricoarytenoid EMG activity (EMGpca); it decreased the duration of EMGdi activity as well as heart rate and mean arterial pressure. In 4-7 day old piglets, preliminary results with naltrindole at an optimal dose of 4 mg/kg showed an increase in the interval between EMGpca and EMGdi activation and in heart rate. From these results we conclude that the mu opioid system plays a greater role in influencing respiration than does the delta opioid system. (Supported by NIH HL36939 and HL07362).

436.13

DIFFERENTIAL RESPONSE TO CYANIDE AND HYPOXIA OF ADULT CAROTID BODY GLOMUS CELLS. D.F. Donnelly, T.R. Cummins and G.G. Haddad. Dept. of Pediatrics, Yale Univ. School of Medicine, New Haven, CT 06510

Both cyanide and hypoxia stimulate carotid chemoreceptors, in vivo and in vitro, but the mechanism(s) of this stimulation is obscure. To investigate the transduction process, we examined the membrane currents of glomus cells which were acutely dissociated from adult rabbit carotid bodies. Glomus cells were identified by their morphologic appearance, binding of FITC-a-bungarotoxin and ability to generate action potentials. Cells were bathed in HEPES buffer at 25C and recorded using whole-cell patch technique. During the experimental period, the cells were stepped from -70 mV to 0 mV every 6 sec. Histotoxic anoxia was induced using brief applications (5-20 sec) of NaCN (2-4 mM) in HEPES buffer. Hypoxic hypoxia was induced by application of HEPES buffer equilibrated with nitrogen (PO2<10 mmHg). Cyanide caused a significant increase in late outward current (139±4% of control, mean±SEM, n=12) which occurred in all cells studied. Hypoxic hypoxia caused a significant decrease in the late outward current (mean= 66.4±2.7%, n=9). Neither agent affected voltage-dependent inward current. These results shows that histotoxic hypoxia and hypoxic hypoxia cause changes in the voltage-dependent outward current of glomus cells and suggest a fundamental separation of transduction processes for the two stimulatory agents.

436.14

ULTRASTRUCTURAL ANALYSIS OF POTENTIAL CHEMO/BARO-RECEPTOR CELLS IN THE MIDDLE EAR TYMPANIC PLEXUS OF A PRIMATE. P.J. Gannon[1], K.J. Chandross[2] J.T. Laitman[3*] and A.R Eden[4]. Dept. Otolaryngology, Mount Sinai Med.Ctr.[1,3,4] New York, NY 10029, and Dept. Neurosciences, Albert Einstein Coll. Med.[2], Bronx, NY 10461.

The maintenance of a stable environment in the middle ear air space is critical for efficient transference of airborne sounds to the fluid-filled inner ear and organ of corti. In most mammals (including humans) auditory performance is critically related to individual fitness. As such, evolutionary processes must have been operating under strong selective pressures, especially with the advent of speech in modern humans where auditory-vocal communication became the primary interactive medium.

The neural mechanisms underlying homeostasis of middle ear gas (air) composition and pressures are only just starting to be understood. We have demonstrated neural connections between the middle ear and respiratory brainstem centers which may subserve such a mechanism. Although the circuitry has been established, the identity of the middle ear sensory modality remains unknown. Our work has focussed on a population of cells, the glomus/ganglion cell clusters of the middle ear tympanic plexus, which show some similarities to those of the carotid body/sinus, and may represent an analogous sensory apparatus. We have suggested that the late appearance of these cells during primate development may be related to the extremely high incidence of otitis media in human infants. In the present study we saw degenerating axosomatic synapses on glomus cells, bilaterally, after unilateral SCG'ectomy. The cell clusters, comprising approximately 100-150 cells are thus associated with both sensory and autonomic elements. Ultrastructural analysis did not reveal features which characterize carotid system sensory elements, such as large dense cored vesicles and fenestrated endothelia. However, their function as chemo/ baro-receptors could not be ruled out. For example, the clusters were often associated with an extensive and tortuous vascular bed, suggesting a (middle ear) blood gas/pressure sampling system with perhaps different physiologic characteristics than the carotid system.

436.15

ALTERATIONS OF TOTAL LUNG RESISTANCE (TLR) EVOKED FROM CAUDAL VENTROLATERAL MEDULLA (CVL) AND ROSTRAL VENTROLATERAL MEDULLA (RVL) IN DOGS. J.R. Haselton, P.A. Padrid* and M.P. Kaufman*. Div. of Cardiovascular Medicine, Univ. California, Davis, CA 95616

A previous report from this laboratory demonstrated that chemical stimulation of neuronal cell bodies in CVL, in the dog, resulted in a reduction of TLR due to the withdrawal of cholinergic bronchomotor tone (J. Appl. Physiol., 63:912-917). The RVL was not examined in that study. In the present study, triple-barrel micropipettes were used to explore CVL and RVL for bronchomotor responses. Each barrel was filled with one of the following: 100 mM DL-homocysteic acid (DLH), 0.9% NaCl, or 2% Fast Green. All substances were adjusted to a pH of 7.40 using HCl or NaOH. Microinjections (25 nl) were made in the ventrolateral medulla at levels ranging from the obex to the caudal pole of the facial nucleus. All sites were verified histologically following the experiment.

In CVL DLH, but not saline, elicited a decrease in TLR (8.2±0.7 to 6.7±0.6 cm H_2O/l/s) and a reduction in blood pressure (BP; 133±4 to 81±15 mm Hg) at 5 sites. One site, in the nucleus ambiguus, evoked an increase in TLR (6.6 to 8.1 cm H_2O/l/s) a reduction in BP (124 to 66 mm Hg) and a profound bradycardia (134 to 2 bpm). In RVL DLH, but not saline, produced: a) an increase in TLR (6.4±0.5 to 8.7±1.2 cm H_2O/l/s) and BP (120±9 to 164±15 mm Hg) at 9 sites, b) a decrease in TLR (8.8±0.5 to 8.0±0.5 cm H_2O/l/s) and increase in BP (138±4 to 161±6 mm Hg) at 3 sites, or c) an increase in BP (125±11 to 143±9 mm Hg) without a change in TLR at 5 sites. The RVL sites, from which an increase in TLR was elicited, were consistently placed ventral to the nucleus ambiguus. Pressor sites which did not elicit a change in TLR as well as those which evoked a decrease in TLR were located either medial (n=7) or lateral (n=1) to those which evoked an increase in TLR. The increase in TLR elicited from RVL was not altered following propranolol (1.5 mg/kg; iv) but was abolished by atropine methyl nitrate (1 mg/kg; iv). We conclude that CVL inhibits, while RVL activates, cholinergic drive to the airways.

436.16

ROLE OF CAPSAICIN-SENSITIVE BARO- AND CHEMO-AFFERENTS IN THE CONTROL OF BLOOD PRESSURE AND RESPIRATION. G. Daniell* and T. R. LaHann. College of Pharmacy, Idaho State University, Pocatello, ID 83209

Although capsaicin inactivates small diameter sensory neurons, most reports suggest that capsaicin pretreatment has little or no effect on blood pressure or respiration. This implies that unmyelinated afferents are unlikely to be important for control of respiratory or cardiovascular function. Our results do not entirely support this interpretation. We have investigated the effect of local application of capsaicin on baro- and chemo-afferent function. Capsaicin or its vehicle was applied to the vagus, carotid sinus and glossopharyngeal nerves. Baroreflex function was evaluated 2 hours post treatment in unconscious rats and 24 hours post treatment in conscious, freely moving rats. Neither capsaicin nor its vehicle altered the relationship between pressor responses and bradycardia in conscious rats. In anesthetized rats, capsaicin pretreatment altered the nature of the baroreflex response without attenuating it. Local application of capsaicin to anesthetized rats also blocked the tachypneic response to stimulation of carotid chemoreceptors (evoked by i.v. cyanide) and eliminated chemoreceptor-induced pressor responses. We interpret these results to mean that capsaicin-sensitive chemoafferents may be important in the control of respiratory function, but that capsaicin-sensitive baroafferents are unlikely to modulate baroreflex function in conscious animals under low stress conditions.

436.17

TRIGEMINAL INJECTIONS OF LIDOCAINE INHIBIT THE CARDIORESPIRATORY RESPONSES TO STIMULATION OF THE SUPERIOR LARYNGEAL NERVE IN THE MUSKRAT. W.M. Panneton. Dept. of Anat. & Neurobiol., St. Louis Univ. Sch. of Med., St. Louis, MO 63104.

Electrical stimulation of the superior laryngeal nerve (SLN) induces apnea and bradycardia, similar to the responses seen after stimulation of the nasal cavity with ammonia. The SLN contains primary afferent fibers which project to both the nucleus tractus solitarii and the caudal trigeminal complex (MDH). The purpose of this study was to determine if blocking the trigeminal projection affects the cardiorespiratory responses to SLN stimulation.

Muskrats were anesthetized, their SLN isolated bilaterally, and placed on hook electrodes. Respirations, heart rate and arterial pressure were monitored on a physiograph. Bilateral injections (100-200nl) of 2% lidocaine were placed in the MDH and the SLN stimulated (0.5-4mA; 10-40Hz; 5s). When both the lidocaine injections were placed in ventral parts of the rostral MDH, especially those centered in laminae I and II, the apnea and bradycardia usually elicited by SLN stimulation were reversibly inhibited. These data suggest trigeminal neurons are important for the cardiorespiratory responses after SLN stimulation. Moreover, the rostral MDH may modulate the autonomic responses to stimulation of the whole upper respiratory tract since similar injections inhibit the responses seen after nasal stimulation. Supported by NIH grant HL 38471.

436.18

DETERMINISTIC BREATHING PATTERNS AS REVEALED BY RECURRENCE PLOTS OF DYNAMICAL SYSTEMS. Charles L. Webber, Jr. and Joseph P. Zbilut*. Department of Physiology, Loyola University of Chicago, Maywood, IL 60153 and Departments of OR/Surgical Nursing and Physiology, Rush Medical College, Chicago, IL 60612.

Several methods have been devised for the computation of dynamical parameters from time series (correlation dimension, entropy, Liapunov exponents, etc.), all of which assume stationarity in the original data. However, another powerful technique of nonlinear dynamics has been introduced, the recurrence plot, which reportedly has the ability to discriminate subtle time-correlation information in time-varying data that are otherwise inaccessible (Europhys. Let. 4:973-977, 1987). To test the utility of this remarkable graphical tool on a physiological system with inherently adiabatic (slowly changing) properties, intrathoracic pressures were recorded from unanesthetized rats during daytime sleep and wakefulness. Derived respiratory cycle times were used to generate recurrence plots by placing points on a square array whenever two points were within a specified distance of each other depending upon the selected embedded dimension. The results revealed deterministic patterns in the respiratory controller, especially in the awake state, which were comparable to standard mathematical chaotic systems (Henon attractor, quadratic difference equation). It is concluded that recurrence plots are very valuable in diagnosing subtleties in dynamical systems.

REGULATION OF AUTONOMIC FUNCTION: CONTROL OF LUMBOSACRAL AUTONOMIC OUTFLOW

437.1

LIMIT CYCLE AND NEGATIVE FEEDBACK IN BLADDER CONTROL FOLLOWING SPINAL CORD INJURY. J.S. Walter, P. Zaszczurynski*, J.S. Wheeler*, R.D. Wurster. Rehab. R&D Center, Hines VA Hosp., Hines, IL 60141.

We have begun to explore models of a bladder control system following spinal cord injury by conducting perturbation analysis and nerve anesthesia/stimulation studies. Periodic bladder contractions occurred in chronic, unanesthetized, spinal-injured, male cats (T-1) when the bladder volume was maintained above the micturition threshold, 30 to 60 ml. A perturbing volume of 3 to 5 ml phase advanced or delayed the bladder contraction depending on whether the volume was added or withdrawn respectively. The period, however, was unchanged. These observations are typical for rhythms controlled by a stable limit cycle system.

In addition to the known positive feedback for bladder control, the presence of negative feedback was indicated in the anesthetized animal by comparing declines in bladder contractions when the pelvic nerves were intact and after direct bladder stimulation with crushed pelvic nerves. In both cases declines in pressure had similar slopes. Therefore, with intact pelvic nerves, declines in bladder pressure during spontaneous bladder contractions may be due to inhibitory processes or "turning off" pelvic motor activity, analogous to turning off our direct bladder stimulator. Supported by Rehab. R&D Center and Merit Review Grant B441.

437.2

ROLE OF CRF AND 5HT IN CENTRAL PATHWAYS CONTROLLING MICTURITION IN THE RAT. T.Suzuki, M.Kawatani, S.Erdman*, W.C.deGroat, Dept of Pharmacology and Behavioral Neuroscience, University of Pittsburgh, Pgh., PA 15261.

Bulbospinal pathways from the pontine micturition center (PMC) and raphe nuclei to the parasympathetic nucleus in the lumbosacral spinal cord of the rat contain CRF and 5HT, respectively, and have been implicated in the central control of micturition. The present study examined the role of these pathways in regulating bladder reflexes (BR) in urethane anesthetized rats. In normal rats distension of the urinary bladder (UB) evoked rhythmic bladder contractions and firing on UB postganglionic nerves. BR were acutely blocked by bilateral electrolytic lesions (2mA,10sec) in the PMC but recorded 7 days after the lesions. Intrathecal (IT) injections (L_1-L_2 segment) of CRF (0.3-30µg) depressed BR. Pretreatment of rats 7 days prior to experiments with 5,7-dihydroxytryptamine (100µg,IT) a toxin that destroys 5HT axons did not block BR induced by UB distension or by electrical stimulation of UB afferents, however in 50% of the animals did produce hyperactive UB activity.

These data suggest that pathways from the PMC and from 5HT neurons in the raphe nuclei have facilitatory and inhibitory influences, respectively, on micturition. Since IT CRF inhibits UB activity it seems likely that this peptide is not the transmitter in the descending excitatory pathway from the PMC to the lumbosacral parasympathetic nucleus.

437.3

THE EFFECTS OF MK-801 ON THE MICTURITION REFLEX IN THE RAT - POSSIBLE SITES OF ACTION. M. Yoshiyama, J.R. Roppolo, V. Erickson* & W.C. de Groat. Univ. of Pittsburgh, Sch. of Med., Dept. of Pharmcol. Pittsburgh, PA 15261.

The possible sites of action and effects of MK-801 (1µg-30mg/kg I.V.) on bladder pressure recorded via a transurethral catheter were compared in five preparations: 1) Intact rats 2) Chronic spinal (T6 - T8) rats 3) Unanesthetized decerebrate rats 4) Intact rats tested 17-24 hours following reserpine (5mg/kg I.M.) 5) Intact rats with MK-801 given via intrathecal (I.T.) catheter at the L6-S1 spinal cord segment. Animals were anesthetized with urethan (1.2gm/kg S.C.) except the decerebrated animals, whose surgery and precollicular decerebration was performed under halothane (2.5%) anesthesia. MK-801 (0.3-1mg/kg I.V.) given to intact rats produced complete inhibition of bladder activity. In decerebrate and chronic spinal animals the same and considerably higher doses (3-30mg/kg I.V.) produced little or no effect on bladder activity. Intrathecal administration of MK-801 to intact rats inhibited bladder contractions at a total dose of 12-36 ug. Reserpine pretreatment prevented the inhibition produced by MK-801 in 50% of the animals tested. These data suggest at least two possible sites of action of MK-801: the forebrain rostral to the superior colliculus and the spinal cord. The latter may depend upon descending input from the forebrain. The reduction of MK-801 effects in reserpinized rats suggests that in addition to an action on excitatory amino acid synapses, that there is also an involvement of catecholamine and/or 5HT mechanisms in the action of MK-801.

437.4

REORGANIZATION IN CAT VESICAL GANGLIA FOLLOWING SACRAL ROOT RESECTION A.M. Booth, M. Kawatani, S. Smerin, & W.C. de Groat Depts. of Pharmacol. & Behav. Neurosci. Univ. of Pittsburgh School of Medicine, Pittsburgh, PA 15261

Transection of the sacral parasympathetic preganglionic input to the urinary bladder leads to: 1) abnormal sympathetic activation of the bladder 2) unusual patterns of spontaneous and stimulus evoked activity in bladder ganglia (BG) and 3) altered sensitivity of BG to substance P (SP). These findings illustrate the plasticity in BG following chronic partial decentralization. This study utilized extra and intracellular recording to examine decentralized BG (DBG) invitro and insitu 15 weeks to 15 months after lesioning. In > 100 cells in DBG the % of cells exhibiting synaptic responses to electrical stimulation (ES) of preganglionic nerves (PGN) was smaller (35-65%) than in normal BG (85-100%) while the % of cells in DBG exhibiting spontaneous firing was much higher (25-55% vs 0-5%). Many cells exhibited 2-10/sec, large amplitude (2-20 mV) "spontaneous" epsps. Repetitive ES (2-50Hz) of PGN elicited an increase in spontaneous activity that was positively correlated with the frequency and duration of the ES. Cells in DBG received fewer inputs (2-4 vs 6-7) and while threshold for spike initiation was unchanged (8-15 mV) the probability of eliciting firing was greatly increased. Both SP and VIP evoked asynchronous firing at doses that were ineffective in normal BG. We conclude that synaptic plasticity in DBG can markedly change the efferent input to the bladder and may contribute to the development of the autonomous hyperactive bladder. (Supported in part by NS-22436, NS-25254 & AM-37241)

437.5

BLADDER CONTRACTIONS EVOKED BY MICROSTIMULATION OF THE SACRAL SPINAL CORD J.R. Roppolo, A.M. Booth, S. Smerin, I. Nadelhaft & W.C. de Groat Dept. of Pharmacol. Univ. of Pittsburgh School of Medicine, Pittsburgh, PA 15261

This study examined the effect of focal electrical microstimulation of the sacral spinal cord on bladder activity. In intact adult male chloralose anesthetized cats, the bladder was cannulated for pressure recording and a laminectomy exposed the lumbosacral spinal cord (L_3-S_3) and roots. The sacral segment containing bladder preganglionic neurons (PGN) was identified by stimulation of each ventral root (10-15 Hz, 0.05 sec pulse). S_2 root stimulation produced maximal responses (60-100 cm H_2O). The S_2 cord was then stimulated (15Hz, 10 sec duration, 0.2 msec charge balanced pulses, 5-25 uA) with a tungsten microelectrode (8-10 µm exposed tip) directed toward the PGNs of the sacral parasympathetic nucleus (SPN). Bladder contractions (maximum amplitude of 20-40 cm H_2O) were evoked over a distance of 0.5-0.8 mm in the intermediolateral gray (1.5-2.3 from surface) and were not usually associated with gross somatic motor activity. stimulation dorsal or ventral to the SPN could evoke bladder contractions, but thresholds were higher and skeletal muscle responses prominent. Bladder contractions were abolished with ganglionic blockade (trimethaphan 0.25 mg/kg) but were unchanged following neuromuscular blockade (pancuronium 0.1 mg/kg). These studies demonstrate that bladder contractions can be evoked selectively by focal electrical stimulation of the sacral spinal cord. (Supported by NIH contract NO1-NS-9-2366)

437.6

POSTNATAL DEVELOPMENT OF THE SENSORY INNERVATION OF THE URINARY BLADDER IN THE RAT. M.N. Kruse, M. Tanowitz*, C.Cheng* and W.C. de Groat. Depts. of Pharmacology and Behavioral Neuroscience, Univ. of Pittsburgh, Pittsburgh, PA 15261 and National Defense Medical Center, Taiwan.

The bladder-to-bladder reflex does not become functional in the neonatal rat until ~ day 6. It has been suggested that the absence of the bladder-to-bladder reflex at birth is due to lack of maturation of the sensory afferents from the bladder. This study addressed the question of whether the neonatal rat is born with an intact sensory pathway from the bladder to the spinal cord. Wheat germ agglutinin-horseradish peroxidase (WGA-HRP) was crushed into the urinary bladder of pentobarbital or halothane anesthetized neonatal rats (day 0 to day 8). The spinal cords and dorsal root ganglia (DRG) were removed 10-48 hours later for TMB processing. WGA-HRP labelled cells were seen in the L_6 DRG (~75%) and S_1 DRG (~25%) in day 0 to day 9 pups. WGA-HRP labelling was also seen in the sacral parasympathetic nucleus and lateral dorsal horn of the L_5, L_6 and S_1 spinal cord of day 0 through day 9 pups. These data indicate that bladder afferent neurons at birth have a segmental distribution and central projections similar to that of adults. The lack of a functional bladder-to-bladder reflex before postnatal day 6 could be due to immaturity of afferent synaptic mechanisms or of interneuronal pathways in the CNS.

437.7

NEUROBIOTIN (NB) and HRP DEFINED DENDRITIC MORPHOLOGY SUGGESTS ADDITIONAL FUNCTIONS FOR SACRAL PREGANGLIONIC NEURONS (PGN) **S.J. Zhang*, L.A. Felkins*, W.C. de Groat, and C.W. Morgan** Depts.Anatomy and Neurobiology and Urology, Eastern Virginia Medical School, Norfolk, VA 23501; Dept. Pharmacology, Univ.Pittsburgh, Pittsburgh, PA 15261

PGN in the lateral band (LB) of the sacral parasympathetic nucleus of the male cat are presumed to subserve various urogenital functions. Intracellular labelling was performed on more than 20 LB neurons with B-fiber axons to determine whether different subpopulations could be distinguished morphologically. At least five types of neurons were identified based on combinations of dendritic distributions in (1) the lateral funiculus, (2) lamina I, (3) the dorsal commissure and (4) the ventral horn. Groups of cells had dendritic projections to regions: 1,2,3 & 4; 1&4; 1,3 & 4; 2,3 & 4 or 3&4.

These data suggest that the functions of sacral lateral band PGN (bladder, penile, urethral, seminal vesicles) might be correlated with and predicted by their dendritic patterns. Further studies are underway to directly test this hypothesis by identifying the function of cells prior to intracellular labelling.

Supported by NINDS RO1 NS26585

437.8

NEURONS LABELLED FOLLOWING THE APPLICATION OF TRACERS TO THE DISTAL CUT HYPOGASTRIC NERVE OF THE RAT. I. Nadelhaft and P. L. Vera. VA Med Ctr & Depts of Neurosurg and Pharmacol and Center for Neurosci, Univ of Pittsburgh, Pittsburgh, PA.

Following the work of Baron and Janig [JANS 24:81('88)] in the cat, we applied dyes to the distal stumps of the transected hypogastric nerves (HGN) of the female rat. The animal was anesthetized with halothane and one hypogastric nerve dipped into fast blue and the other into fluorogold. After about 10 days, the animal was reanesthetized with pentobarbital and perfused with 4% buffered paraformaldehyde. Labelled neurons numbering in the hundreds were located mainly ipsilaterally; however there were significant numbers of contralateral cells present. In the major pelvic ganglion (MPG) cells were concentrated within a small area near the entrance of the hypogastric nerve. Approximately one half of these stained positively for tyrosine hydroxylase. Labelled cells were located in the T13-L3 (35%) and L6-S1 (65%) dorsal root ganglia. In the sympathetic chain, labelled neurons were found between T12 and S2. A few preganglionic neurons were found in spinal cord segments L6 and S1. These groups of neurons may be innervating portions of the reproductive system served by small nerve branches leaving the HGNs between the inferior mesenteric ganglia and the MPGs.

437.9

SYMPATHETIC POST-GANGLIONIC INNERVATION OF THE RAT URINARY BLADDER. Pedro L. Vera and Irving Nadelhaft. VA Medical Center, Depts of Pharmacology and Neurosurgery, University of Pittsburgh, Pittsburgh, PA 15240.

Female rats were anesthetized with halothane and the urinary bladders exposed. Fluorescent traces (Fast Blue or Fluoro-Gold) were injected into the bladder wall using a Hamilton syringe. One to 3 weeks after the injections the rats were anesthetized and perfused with phosphate buffered-4% paraformaldehyde. The major pelvic ganglia (MPG), the inferior mesenteric ganglion (IMG) and the sympathetic chain (T10-S3) were removed, sectioned (20 um) in a cryostat and examined for the presence of retrogradely labeled cells. The sections from the MPG were also processed for DBH-immunohistochemistry.

In the MPG, many retrogradely labeled cells were present and some also contained DBH immunoreactivity. In addition, retrogradely labeled cells were found in the IMG. The sympathetic chain contained most of the retrogradely labeled cells. Experiments are in progress to determine the contribution of the hypogastric and pelvic nerves to the sympathetic chain innervation of the urinary bladder of the rat.

437.10

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

The afferent and efferent pathways to the cat ischiocavernosus muscle (ICM) were identified using the retrograde tracers HRP, WGA-HRP, and cholera toxin B-HRP injected into the ICM under halothane anesthesia. 72 to 96 hours later anesthetized cats was perfused with fixative. Spinal cord and dorsal root ganglia (DRG) from L_6 to S_3 were processed with TMB to reveal HRP. Lower motoneurons (average, 150) projecting to the ICM were present exclusively in Onuf's nucleus (ON), in L_7 and S_1. The neurons were typically elongate ranging from 25x30 to 40x70 microns in size. The neurons sometimes formed clusters, but were not confined to any particular division of ON. Cholera toxin B-HRP revealed dendrites up to one millimeter long extending from the ventrolateral division of ON to the medial ventral horn, the lateral funiculus, and the sacral parasympathetic nucleus. Labeled DRG cells (~1000 per cat) present in S_1 and S_2 ranged from 10x20 to 80x110 microns in size. Sparse afferents extended through the medial and lateral collateral pathways into the dorsal gray commissure. The distribution of neurons innervating the ICM overlaps with that of the striated urethral and anal sphincters, suggesting that coordination of the three may take place in the sacral spinal cord. (Supported by NIH contract N01-NS-9-2366)

437.11

INNERVATION AND FUNCTION OF THE ISCHIOURETHRALIS MUSCLE OF THE RAT. W. G. Dail, B. D. Sachs, G. Walton* and Y-C Liu*. Dept. of Anatomy. Univ. New Mexico Sch. of Med., Albuquerque, NM 87131 and the Dept. of Psychol. University of Conn., Storrs CT 06269-1020

The ischiourethralis, a striated perineal muscle presumed to be involved in sexual reflexes, was studied in the rat. The paired muscle arises from the penile crus and penile bulb and unites in a raphe over the dorsal vein of the penis. This structure is identified as the subpubic ligament in other descriptions. Retrograde tracing studies show that the muscle is innervated by neurons in the dorsolateral nucleus of the lumbar spinal cord, a pudendal nerve motor nucleus which also projects to the ischiocavernosus muscle. Excision of the ischiourethralis muscle did not interfere with the ability of males to display normal copulatory behavior, nor did it affect significantly the number and intensity of reflexive erections. The function of this muscle like that of some other perineal muscles remains to be determined.

437.12

A ROLE FOR 5-HYDROXYTRYPTAMINE IN MEDIATING SPINAL SEXUAL REFLEXES L. Marson and K. E. McKenna Dept. of Physiology, Northwestern Medical School, Chicago IL 60611

We previously identified a brainstem site in the ventromedial rostral medulla that mediates the inhibition of spinal sexual reflexes. The location of this site corresponds to the paragigantocellular reticular formation (PGi). The present study provides neuroanatomical and pharmacologic evidence for 5-hydroxytrypamine (5-HT) in the mediation of this inhibition.

Rhodamine-labelled beads were pressure injected into L5-L6 of the spinal cord. After 7-14 days rats were perfused with 4% paraformaldehyde solution. The spinal cord and medulla were cut (30-40μm) and processed for 5-HT-immunoreactivity using the immunofluorescent technique. Retrogradely labelled neurons were found in the PGi, raphe pallidus, raphe obscurus and gigantocellular reticular formation after injections located to the pudendal motoneurons. Many of these retrogradely labelled cells in the PGi contained 5-HT-immunoreactivity. Dense 5-HT-immunoreactive fibers and presumptive terminals were found surrounding the pudendal motoneurons and interneuronal spinal grey of the lumbosacral spinal cord. Male rats were anesthetized with urethane and an intrathecal catheter was inserted into the subarachnoid space, positioned with the cannula tip at L5-L6 of the spinal cord. The rats were spinalized. The coitus reflex was elicited via a catheter inserted into the urethra. The reflex was monitored by EMG recordings of the bulbospongiosus muscle. Microinjection of 5-HT (1-2.5μg) resulted in an increased sensory threshold and delayed the coitus reflex. Injection of higher doses of 5-HT (5-50μg) resulted in inhibition of the previously evoked coitus reflex. Thus, 5-HT appears to alter the spinal pattern generator for the coitus reflex.

These findings provide evidence that 5-HT neurons in the rostral medulla mediate inhibition of spinal sexual reflexes.

437.13

MODULATION OF THE THRESHOLD FOR FICTIVE SEXUAL CLIMAX IN FEMALES BY PERIPHERAL SEROTONIN

K.E. McKenna and Kelley Knight* *Dept. of Physiology, Northwestern Medical School, Chicago, IL 60611*

We previously identified urethral stimuli as sufficient for eliciting fictive sexual climax in anesthetized, spinalized male and female rats (Neurosci. Lett. 94, 343, 1988). While simple mechanical stimuli alone are effective, some observations have led us to consider that urethral chemoreceptor mechanisms may play a role in eliciting sexual climax. The present study was designed to identify the substrates of urethral chemoreception.

Other investigators have shown in a number of species that the urethral mucosa contains serotonergic paracrine cells. The morphology of these cells suggests a chemoreceptor function. To determine if these paracrine cells are present in the rat, male and female rats were anesthetized and perfused with fixative. The urethra was removed and either 50 μm transverse sections cut, or a wholemount preparation of the urethral mucosa was prepared. Immunofluorescent staining for serotonin revealed a large number of small (< 10 μm) heavily stained cells throughout the urethra. There was a concentration of these cells in the urethral bulb/distal urethra. This is the area which we have identified as most sensitive in eliciting fictive sexual climax. These cells have processes which extent to the luminal surface. In the submucosa, the cells displayed one or two large clublike processes.

To examine the physiological role of these cells, female rats were anesthetized, spinalized and a urethral catheter inserted via the bladder. Distension of the urethra by infusion of saline through this catheter followed by urethral meatus occlusion was effective in eliciting the fictive sexual climax, as monitored by pudendal nerve recordings. The pressure threshold required to elicit the fictive sexual climax was highly consistent in a given animal. The addition of serotonin to the infusion fluid significantly and reversibly decreased (by 20 - 30%) the pressure required to elicit the response. The maximally effective dose was 10^{-6} M.

437.14

MAINTENANCE OF ERECTION OF PENILE GLANS, BUT NOT PENILE BODY, AFTER TRANSECTION OF RAT CAVERNOUS NERVES. B.D. Sachs and Y.-C. Liu*, Dept. of Psychology, Univ. of Connecticut, Storrs, CT 06269-1020.

A role for the cavernous nerve (CN) in erection of the corpora cavernosa has been well documented. Evidence for CN-innervation of the corpus spongiosum implies a role in erection of the glans penis as well. However, stimulation of the rat CN led only to erection of the penile body, not the glans (Quinlan et al., J. Urol., 141:656, 1989). To further determine the role of the CN in glans erection, we tested male rats for mating behavior and sexual reflexes after CN transection (CNx). During copulation, the intromission rate of CNx males was drastically reduced, reflecting their impaired ability to erect the penile body. In reflex tests, CNx males continued to display weak and moderate glans erections, but lacked penile body erections and intense glans erections. These results suggest that at least in rats the innervation of the corpus cavernosum differs from that of the corpus spongiosum, and that the CN may play little or no role in corpus spongiosum function.

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437.15

NORMAL SEXUAL REFLEXES IN THE ABSENCE OF URETHRAL STIMULATION IN INTACT COPULATING RATS.

G.M. Holmes, D.H. Lynch* and B.D. Sachs. Dept. of Psychology, Univ. of Connecticut, Storrs, CT 06269.

The absence of seminal and prostatic fluids does not alter the motor patterns or parameters of copulation, including the ejaculatory response (ER), in rats (e.g., Tisell & Larsson, Invest. Urol. 16:274, 1979). Research on anesthetized spinal rats suggested a role for urethral stimulation in the ER, as measured by the EMG of the bulbospongiosus muscle [mBS] (Chung et al., Neurosci. Lett. 94:343, 1988). In order to help place this finding in a natural behavioral context, we tested for its generality in copulating rats. A diagnostic component of the rat ER is high-energy EMG activity in the mBS starting ca. 500 ms after penile insertion and continuing for several seconds after dismount. Anesthesia of the urethral mucosa with lidocaine did not disrupt this mBS response. Systemic injection of guanethidine eliminated ejaculatory plugs but had no effect on the ER. These results suggest that urethral stimulation by the ejaculate does not contribute to the regulation of the striated-muscle components of ejaculation-related reflexes in copulating male rats.

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437.16

FUNCTIONAL PLASTICITY IN DECENTRALIZED AUTONOMIC GANGLIA: LACK OF EVIDENCE FOR MAST CELL INVOLVEMENT. N. Minorsky, G. Walton* and W. G. Dail, Dept. of Anatomy, Univ. New Mexico Sch. of Medicine, Albuquerque, NM 87131.

Stimulation of the hypogastric nerve in the rat results in penile vasodilation only when the pelvic nerve is interrupted. The mechanism for this response is unknown but in view of the hyperemic status of denervated ganglia and the recent suggestion that mast cell products increase excitibility of autonomic ganglion cells, we have compared the number of mast cells in intact and partially denervated major pelvic ganglia (MPG). The number of intraganglionic mast cells (572 ± 109; average ± SEM/MPG) was unchanged when intact ganglia were compared to ganglia in which the pelvic nerve was interrupted seven days earlier. No difference was seen between control and denervated ganglia when an arbitrary scale was used to assess mast cell degranulation. Although it remains possible that mast cells could alter ganglionic transmission following injury, the present study did not find changes in mast cell number and activity coincident with functional plasticity.

437.17

SENSORY INNERVATION OF THE RAT KIDNEY AND URETER AS REVEALED BY THE ANTEROGRADE TRANSPORT OF WGA-HRP FROM DORSAL ROOT GANGLIA. S. F. Echtenkamp and C. F. Marfurt. Northwest Center Med. Educ., Indiana Univ. Sch. of Med., Gary, IN 46408.

The afferent innervation of the rat kidney and ureter was studied by labeling the sensory nerve fibers with wheat germ agglutinin-horseradish peroxidase (WGA-HRP) transported anterogradely from dorsal root ganglia. In some cases, the tissues were serially sectioned at 40µm in a cryostat, whereas in other animals the ureter, renal pelvis, and arterial tree were dissected free of surrounding tissues and processed as whole mounts. Labeled nerve fibers were numerous in the ureteral wall and lining of the renal pelvis. In the ureter, they formed a dense, branching plexus oriented parallel to the long axis of the organ, however, at the widest part of the renal pelvis many of the fibers changed direction and traveled within the lining of the funnel-shaped renal pelvis in a circumferential fashion. The latter axons were of extremely fine diameter and appeared to terminate exclusively as free nerve endings. Other fibers reached the renal pelvis by coursing along branches of the renal artery and then "doubling back" to reach the pelvis along proximal segments of the renal calyces. More modest networks of labeled axons were observed around branches of the renal artery and, to a much lesser extent, the renal vein. Labeled fibers were never seen around afferent or efferent arterioles or within glomeruli. A few fibers entered the renal cortex and terminated in close proximity to renal tubules. This study reveals an anatomical configuration of ureteral and renal pelvic sensory nerves consistent with a role in detection of ureteral pressure changes, as well as a renal artery and vein sensory innervation that may monitor changes in renal vascular pressure.

437.18

URINE RETENTION DUE TO INTRASPINALCORD INJECTION OF COLCHICINE: THERAPEUTIC EFFECT OF BREMAZOCINE, NERVE GROWTH FACTOR AND MONOSIALOGANGLIOSIDE. M.Baraldi, P.Zanoli*, C. Truzzi*. Chair of Pharmacology and Pharmacognosy, School of Pharmacy, Modena University, 41100 Modena, Italy.

We described that the intraspinalcord (ICS) injection of low doses (2-5 ug/rat) of colchicine (C) induces a massive urine retention, bladder hypertrophy and urine overflow. Pharmacological challenges with Bethanecol or Propranolol did not trigger bladder voiding. Neuropeptides have been show to play a key role in the regulation of the micturition reflex in the spinalcord and bladder function. In rats with C-induced urine retention we found at the site of injection an increase of Met-enkephalin, a marked decrease of Substance P and no apparent changes in the level of Dynorphin A. The urine retention was worsened by Naloxone whereas was prevented by Bremazocine, a K and sigma receptor agonist. In the spinalcord of rats with urine retention we detected an increased presence of K and sigma receptors. It seems likely that Bremazocine, despite its K stimulatory activity, by stimulating sigma receptors present on the Mg-sensitive NMDA-glutamate receptors, allosterically inhibits an increased NMDA receptor activity. An implication of a glutamatergic dysfunction in the C-induced urine retention seems to be confirmed by the ability of Nerve Growth Factor and Monosialoganglioside (GM-1), which minimise the neurotoxicity of glutamate, to normalise urine output in C-treated rats.

NEUROENDOCRINE REGULATION: OXYTOCIN, VASOPRESSIN

438.1

PROPERTIES OF POSTERIOR PITUITARY PRIMARY CULTURES. C. Dvergsten*, A.K. Salm, R.B. Meeker, L. Rietz*, R.S. Greenwood and J.N. Hayward. Depts. of Neurology, Pediatrics, and Pharmacology and Neurobiology Curriculum, University of North Carolina, Chapel Hill, NC 27599.

Pituicytes were cultured from the neural lobe of the rat pituitary at developmental ages E16-PN14 and adults. Cultures were grown from both punches of the isolated neural lobe and dissociated cells. The earliest age at which cultures were effectively grown was E17-E18. Cells from E18 fetuses exhibited process bearing and flat-polygonal morphologies, similar but not identical to cultured astroglia. The process-bearing cells exhibited strong GFAP immunoreactivity whereas the flat-polygonal cells had variable levels of staining ranging from robust to weak. As the neural lobe developed, fewer process-bearing cells were observed in culture, until by PN5-7, cultures were largely comprised of flat-polygonal cells and a few small flat cells with or without irregular processes. Cultures of neural lobe punches at PN5-14 contained a core of flat cells surrounded by spindle-shaped, fibroblast-like cells whereas, dissociated cells yielded more homogeneous cultures that were 70-90% GFAP immunoreactive. A majority of the cells also stained robustly for fibronectin. A subpopulation of cells from cultured neural lobe were found to express specific vasopressin binding sites. Stimulation with 10^{-5} M vasopressin, dynorphin and norepinephrine resulted in increased intracellular calcium in subsets of flat-polygonal unidentified cells. Oxytocin and leu-enkephalin had no effect on intracellular calcium concentration.

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438.2

PRIMARY CULTURES OF MAGNOCELLULAR NEUROENDOCRINE CELLS FOR IN VITRO PATCH CLAMP STUDIES AND ANATOMICAL ANALYSES. S.A. Oglesby, L. Rietz*, E. Cox*, R.B. Meeker, R.S. Greenwood and J.N. Hayward. Depts. of Neurology and Pediatrics, and Curriculum in Neurobiology, Univ. of North Carolina, Chapel Hill, NC 27599 and Cato Research Ltd., Durham, NC 27713

To identify optimal culture conditions for physiological studies of magnocellular neuroendocrine cells, the properties of fetal and early postnatal, anterior hypothalamic cultures containing supraoptic and paraventricular neurons, were evaluated under different conditions of tissue preparation and culture. Tissue punches through the region of the supraoptic nuclei, coronal slices or dissociated cells were cultured on coverslips coated with either poly-L-lysine, collagen (Vitrogen) or solubilized tissue basement membrane (Matrigel). The growth characteristics of magnocellular neurons differed under various conditions. Slices and tissue punches produced a rim of healthy neurons which slowly migrated short distances from the cell mass. The migration and axonal outgrowth appeared to be limited to the region of glial outgrowth from the mass. In contrast, a collagen matrix supported rapid axonal outgrowth from cells which extended past the glial outgrowth. This latter type of preparation appears to be best for axonal outgrowth but was less useful for patch clamp studies. Large cells with a morphology similar to immunocytochemically identified magnocellular neuroendocrine cells were easily identified in dissociated cultures. These cells could be patch clamped and infused with Lucifer Yellow. These cultures provide a means by which vasopressin and oxytocin magnocellular neurons can be cultured for different growth properties, studied using standard patch clamp methods, marked intracellularly with Lucifer Yellow, and identified immunocytochemically.

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438.3

SOMATOSTATIN-28(SS-28):CNS SITES AND MECHANISM OF ACTION TO STIMULATE VASOPRESSIN SECRETION. M. Brown and K. Carver-Moore
Department of Medicine, University of California, San Diego, CA 92103.

SS-28 injected into the cerebroventricle of the rat elicits an increase of the plasma concentration of AVP. Studies have been performed using awake rats to determine the neuroanatomic site and mechanism of action of SS-28 to increase plasma AVP levels. SS-28 injected into the central nucleus of the amygdala, the lateral, posterior and supraoptic nuclei of the hypothalamus and several brainstem regions did not alter plasma levels of AVP. SS-28 injected into the bed nucleus of the stria terminalis or the anterior or paraventricular nucleus of the hypothalamus resulted in elevation of AVP greater than those levels observed following administration of peptide into the third ventricle. Two types of mechanisms of action of SS-28 to stimulate AVP release were evaluated: (1) SS-28 stimulation of an endogenous neurotransmitter that acts as an agonist of AVP release and (2) SS-28 inhibition of the release of a neurotransmitter that tonically inhibits AVP release. Antagonists of muscarinic, atropine, and nicotinic, hexamethonium, cholinergic receptors and of angiotensin-II did not alter SS-28 induced AVP release. In contrast, both GABA and isoproterenol attenuated SS-28 induced AVP release. Based on the inhibitory actions of GABA and norepinephrine on AVP release, it is possible that SS-28 acts with the hypothalamus and other brain regions to stimulate pituitary AVP release through inhibition of the release of one or both of these neurotransmitters.

438.4

RAPID VASOPRESSIN mRNA EXPRESSION IN THE RAT HYPOTHALAMUS DURING POLYETHYLENE GLYCOL-INDUCED HYPOVOLEMIA. R.B. Meeker, E. Perkins*, M.M. Nicolle, L. Rietz*, R.S. Greenwood and J.N. Hayward. Dept. of Neurology and Pediatrics and Neurobiology Curriculum, University of North Carolina, Chapel Hill, NC 27599.

The study of the control of vasopressin mRNA expression in magnocellular neurons is hampered by the slow rate of blood volume or osmolality changes following most experimental manipulations, such as water deprivation or hypertonic saline ingestion, which may be rate limiting. Consequently, we characterized the increase in VP mRNA levels by a single subcutaneous injection of polyethylene glycol (PEG) to assess the time course of VP mRNA increases in the supraoptic and paraventricular nuclei in a response to a more rapid challenge. A 30mer oligonucleotide probe specific for vasopressin mRNA was hybridized to 20 um sections of rat brain 0, 4, 8 or 16 hrs after PEG injection or 24 hrs after injection in rats which were allowed to regulate their fluid volume by drinking. Levels of VP mRNA increased within 4 hrs of PEG injection to values approximately, 2-fold greater than control values. The similarity of this time course to the evoked release of vasopressin suggests that the two processes may be under similar transsynaptic control. This paradigm may provide a simple and useful model for in vivo pharmacological analysis of pathways which control vasopressin gene transcription.

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438.5

Stereotactic instillation of morphine into the posterior pituitary of rats in vivo inhibits release of vasopressin. Eric Stephanian, M.D. and Alan G. Robinson, M.D., Departments of Neurosurgery and Medicine, University of Pittsburgh, Pittsburgh, PA 15261

Opioids are reported to act directly on the posterior pituitary to inhibit release of neurohypophysial peptides, but this has been difficult to study with ventricularly administered drugs in intact animals because of the multiple potential sites of action of opioids throughout the central nervous system. We developed a technique for stereotactic cannulation of the posterior pituitary of rats to directly instill substances into the matrix of the neurohypophysis. After cannulation under anesthesia with equithesin basal vasopressin levels in plasma were 10.8 ± 2.5 pg/ml. When a 5 μl solution of 56 mM potassium chloride was infused slowly into the substance of the gland, plasma vasopressin peaked at 83 ± 23 pg/ml at 5 minutes, and gradually returned to baseline over 2 hours. Injection of a solution containing 5.6 mM potassium had minimal stimulatory effect on vasopressin release with peak values of 30 ± 9 pg/ml at 5 minutes. When 56 mM potassium solution was injected concurrently with 5 μg morphine in the same 5 μl solution, vasopressin release was inhibited with a value of 33 ± 3 pg/ml at 5 minutes, a slight rise at 15 minutes and return to basal. Injection of morphine peripherally at the same concentration had no effect on the release of vasopressin during high potassium stimulation. Thus, we have used an in vivo technique to document that opioids delivered to the neurohypophysis directly inhibit the release of vasopressin independent of action elsewhere in the central nervous system.

438.6

FOS EXPRESSION DEMONSTRATES HETEROGENEITY OF MAGNOCELLULAR VASOPRESSIN AND OXYTOCIN RESPONSE. M.M. Roberts, A.G. Robinson, M. Fitzsimmons, and G.E. Hoffman, Departments of Medicine, University of Pittsburgh, Pittsburgh, PA 15261.

Activated vasopressin (AVP) and oxytocin (OT) neurons of the supraoptic (SON) and paraventricular (PVN) nuclei express fos, a proto-oncogene product involved in transcription. C-fos expression was used to map the response of individual magnocellular neurons to graded hemorrhage in rats. Following a 2 cc bleed, 12% of AVP neurons and 17% of OT neurons expressed fos. Of those neurons expressing fos, 38% expressed fos at a low intensity level and only 4% expressed fos at the highest intensity level. After a 6 cc bleed, 92% of AVP neurons and 86% of OT neurons expressed fos; 18% at low intensity and 38% at the highest intensity. As the degree of stimulus increased, both the total number of cells responding and the intensity of response per cell increased. Fos expression in cells double-labelled for AVP or OT delineated hormone-specific differential functional anatomy in the SON and PVN. In the SON, after 2 cc hemorrhage, 15% of AVP neurons, but only 2% of OT neurons were activated. In the PVN, there was no activation of AVP neurons, but 26% of OT neurons were activated. A similar differential response pattern was seen with 4 cc hemorrhage, but with 6 cc most neurons were activated in both nuclei. Fos immunoreactivity thus provides a permanent record of activated neurons in the SON and PVN. Dual immunostaining for fos and AVP or OT was used to demonstrate a previously unrecognized functional anatomic heterogeneity in the threshold for response of magnocellular neurons to hemorrhagic stimuli.

438.7

TOPOGRAPHY OF HYPOTHALAMIC OXYTOCIN NEURONS THAT EXHIBIT c-fos IMMUNOREACTIVITY UPON OSMOTIC STRESS IN THE RAT. Lisa Giovannelli, Priyattam J. Shiromani, Gustav F. Jirikowski and Floyd E. Bloom, Dept. Neuropharmacology, Scripps Clinic Research Foundation, La Jolla CA 92037 and Dept. Psychiatry, UCSD, La Jolla CA 92093.

In order to evaluate the metabolic responses of specific neurons to experimental perturbations in vivo, we have visualized c-fos and oxytocin (OXY) expression in rat brain by double- immunostaining. Both a monoclonal and a polyclonal antibody raised against c-fos peptide sequence 4-17 were used. In normal untreated male rats, or in male rats treated with intraperitoneal injection of isotonic saline, no OXY-immunoreactive neurons were found to express detectable c-fos immunoreactivity. However, nuclear c-fos immunoreactivity was readily detectable in OXY neurons (as well as in other neurons non-immunoreactive for OXY) as early as 90 minutes after intraperitoneal injection of a hypertonic (1.5 M NaCl) salt solution. At this time point neurons immunoreactive for both OXY and c-fos were found in the supraoptic nucleus, in the paraventricular nucleus and in the lateral subcommissural nucleus. These results suggest that oxytocin neurons can be metabolically activated by osmotic stimuli, and indicate the need to further understand the role of the neurons expressing this neuropeptide. Grant n. NS22347

438.8

Water-deprivation increases glucose utilization in the hypothalamo-neurohypophysial system and circumventricular organs. M.Kadekaro, S.Freeman, M.L.Terrell and J.S.Harris. Division of Neurosurgery, University of Texas Medical Branch, Galveston, Texas 77550.

We studied with the quantitative [^{14}C]deoxyglucose autoradiography the neural pathways activated by 24, 48 and 72 h water-deprivation and intravenous infusion of hypertonic saline (2.5 M, 250 ul/min for 4 min) in adult male Sprague-Dawley rats (n=61). Water-deprivation over 72 h induced a progressive increase in plasma osmolality and hematocrit. Hypertonic saline increased plasma osmolality to a larger degree than 72 h water-deprivation (Δ=27 vs.16 mOsm/kg). Hypertonic saline increased glucose utilization in the supraoptic and paraventricular nuclei and neural lobe but not in the circumventricular organs. Glucose utilization increased in the supraoptic nucleus throughout the period of dehydration. In the neural lobe, a tendency to increase started at 24 h of water-deprivation and substantial increments were seen afterwards. In the paraventricular nucleus, subfornical organ (SFO) and organum vasculosum laminae terminalis (OVLT), the increases were observed only after 48 and 72 h dehydration. No changes were seen in the area postrema and median eminence. These results demonstrate that water-deprivation and hypertonic saline stimulate activity of the hypothalamo-neurohypophysial system, but only water-deprivation stimulates activity of the SFO and OVLT.

438.9

ANTERIOR HYPOTHALAMIC (AV3V) LESIONS IMPAIR MAXIMAL SUPPRESSION OF VASOPRESSIN SECRETION IN RATS. R.E. Blackburn, E.M. Stricker and J.G. Verbalis, Departments of Behavioral Neuroscience and Medicine, University of Pittsburgh, Pittsburgh, PA 15261.

Lesions of the antero-ventral third ventricular (AV3V) region severely impair osmotically-stimulated secretion of vasopressin (AVP) and oxytocin (OT) from the rat neurohypophysis. Since suppression of AVP secretion also may involve an osmoreceptor-mediated process, we studied the ability of AV3V-lesioned rats to excrete free water loads as an index of their AVP suppressibility. Radiofrequency lesions were made in male rats (2 mm probe with 2 mm tip exposure; 7.3 mm below bregma in the midline), and the adequacy of the lesion was evaluated functionally by the absence of drinking 1h after induced hyperosmolality (2 M NaCl, 2 ml i.p.). The successfully lesioned rats were evaluated using two different protocols. In one, rats were given 40-70 ml/d of a palatable dilute liquid formula (AIN-76, 1 kcal/ml) as their only source of calories to produce self-ingested water loads. Of 9 rats so treated, 6 became significantly hyponatremic by 2 days after the lesion (plasma [Na^+] = 131.6±2.6 mmol/l, versus plasma [Na^+] = 141.1±0.4 mmol/l in 11 non-lesioned rats on the same liquid formula). A second group of rats were injected i.p. with hypotonic fluid (0.05M NaCl with or without 2.5% dextrose; 15 ml hourly x 4). Plasma [Na^+] of the AV3V- lesioned rats 1h after the last injection was 117.6±3.5 mmol/l (n=6) as compared to 132.8±3.3 mmol/l in sham-lesioned rats (n=5) treated similarly ($p < 0.02$). Our results therefore show that AV3V lesions significantly impair the ability of rats to excrete free water maximally, which most likely reflects non-suppressed basal plasma AVP levels despite plasma hypoosmolality. Consequently, these studies support the hypothesis that both stimulatory and inhibitory components of the osmoreceptor system(s) are located in the AV3V area, and are of importance for complete regulation of neurohypophyseal secretion of AVP.

438.10

OXYTOCIN mRNA LEVELS IN HYPOTHALAMIC PARAVENTRICULAR AND SUPRAOPTIC NUCLEI (PVN/SON) DURING LACTATION IN RATS: EVIDENCE FOR MAINTENANCE BY AFFERENT STIMULI FROM THE OFFSPRING. L. H. Spinolo, R. Raghow*, and W. R. Crowley, Dept. of Pharmacology, University of Tennessee-Memphis, College of Medicine, Memphis, TN 38163.

The objective of the present studies was to describe in detail the changes in oxytocin (OT) mRNA in the PVN/SON that occur over time during pregnancy and lactation in the rat and to test whether afferent stimuli provided by the offspring during lactation influence these levels. The PVN and SON were microdissected from pregnant and lactating females, and RNA was extracted by the RNAzol method. OT mRNA was quantified by slot-blot hybridization, using a 25 mer oligonucleotide probe complementary to bases 912-936 of OT-neurophysin precursor mRNA (Kawata et al., Brain Res. Bull 20: 693-697, 1988), which was T-4 polynucleotide kinase-end labelled, followed by autoradiography. Blots were stripped and then reprobed with an 1125 bp cDNA insert complementary to α-tubulin mRNA for normalization. The levels of OT mRNA in PVN/SON were high on pregnancy day 1, reduced during pregnancy days 7-18, and increased prior to parturition from pregnancy days 20-22. Similar elevated levels were observed throughout lactation. Compared to age-matched controls, immediate removal of offspring on the day of parturition resulted in a marked decline of OT mRNA by 24 hr, and levels tended to remain low for 48-72 hr. Similar results were seen after offspring removal on lactation day 8. These data suggest that OT mRNA levels in the hypothalamic magnocellular regions undergo an initial increase prior to parturition and that the maintenance of these elevated levels during lactation is influenced by afferent stimuli provided by the offspring.

438.11

DNQX BLOCKS BASAL AND OSMOTICALLY STIMULATED VASOPRESSIN RELEASE. C.D. Sladek, Depts of Neurology and Neurobiology and Anatomy, University of Rochester School of Medicine, Rochester, NY, 14642.

In previous experiments, the non-specific excitatory amino acid (EAA) receptor antagonist, kynurenic acid, prevented osmotic stimulation of vasopressin (VP) release from hypothalamo-neurohypophyseal (HNS) explants. In order to evaluate the type of EAA receptor involved in this response, the effect of DNQX (6,7-dinitroquinoxaline-2,3-dione), an antagonist at the kainate and quisqualate EAA receptors was evaluated. VP release was measured from perifused HNS explants exposed to an increase in the NaCl concentration of the perifusate sufficient to increase the osmolality 15 mosmol/kg H2O over 1 hour. DNQX (10μM) or the DMSO vehicle (1.25 μl/ml) were added 60 min prior to the NaCl induced increase in osmolality. DMSO increased osmolality from 290 - 310 mosmol/kg H2O. In the controls, this was associated with a brief increase in VP release ($p<0.05$). The response to the subsequent NaCl induced increase in osmolality was similar to previous experiments with an initial stimulation of VP release, followed by inhibition and a rebound increase in VP release associated with returning the NaCl concentration to baseline. DNQX reduced VP release to 56±9% of basal release ($p<0.05$, n=8), and VP release remained uniformly suppressed during the subsequent NaCl induced increase and decrease in osmolality. These data support the involvement of EAA transmitters in basal and osmotically regulated VP release from HNS explants, and indicate that non-NMDA EAA receptors are important in regulating VP release from the posterior pituitary. Supported by NIH RO1-DK-19761.

438.12

Effect of Extended Exposure to Hypertonicity on Vasopressin mRNA Content of Hypothalamo-Neurohypophyseal (HNS) Explants. C. Yagil and C.D. Sladek. Dept. of Neurology and Neurobiology and Anatomy, Rochester Univ. M.C., Rochester, NY 14642.

VP mRNA content of organ cultured explants of the rat HNS was evaluated following either a step increase in osmolality or a gradual increase of the same amount over 24 hours. Explants were exposed to the step increase in osmolality by placing them directly into hypertonic medium (>304 mosm/Kg H_2O) in static culture. VP mRNA content was significantly decreased in explants maintained for 24 or 48 hours in hypertonic medium compared to explants maintained in isotonic medium (47±10% and 57±6% respectively; $p<0.01$). Basal VP release was also lower in these explants compared to controls, and they did not respond to a further acute increase in osmolality. VP content of the posterior pituitary did not differ between the groups. To achieve a slow increase in osmolality (16 mosm/Kg H_2O over 24 hours by increasing the NaCl concentration), explants were perifused in individual chambers. This stimulus caused a significant increase in VP release which was sustained for more than 9 hours and VPmRNA content was 165±19% higher than control explants ($p<0.001$). No difference existed in VP content in the posterior pituitary. These observations suggest a correlation between VP release and VPmRNA content, because the step increase in osmolality led to lower VP release and lower VPmRNA content in HNS explants where as the slow increase in osmolality caused a sustained increase in VP release and an increase in VP content. Supported by NIH RO1-DK-19761 and the Kidney Foundation of Upstate NY.

438.13

EFFECTS OF ANESTHESIA ON VASOPRESSIN (VP) AND OXYTOCIN (OT) NEURONS IN RESPONSE TO ACUTE SALT LOADING. S.W.T. CHENG* and W.G. NORTH. Dept. of Physiology, Dartmouth Medical School, Hanover, NH 03756.

In a previous study we showed that anesthesia induced by a combina-tion of Ketamine and Nembutal did not significantly influence the func-tion of VP neurons. For this study, we examined the responses of OT and VP neurons to acute salt loading in conscious rats (CON, n=8) and rats under either Nembutal (NEM, 50 mg/kg, ip, n=8) or Urethane (URE, 1.6 g/kg, ip, n=8) anesthesia. Fifteen minutes following the in-duction of anesthesia, NEM produced an increase in basal plasma osmo-lality (Posm, 290 ± 2 to 296 ± 3 mOsm/kg H_2O, $p < 0.007$) while URE did not change basal Posm (287 ± 2 to 289 ± 2 mOsm/kg H_2O). Neither anesthetic resulted in any significant changes in basal plasma levels of oxytocin-associated neurophysin and vasopressin-associated neurophysin (OT-RNP, VP-RNP). In response to intravenous infusion of 18% saline, all three groups of rats had similar rises in Posm. The slopes of the relationship between plasma OT-RNP and Posm were not significantly different between the CON and the NEM groups, while that for the URE group was significantly ($p<0.05$) smaller than the CON group (CON=10.9 ± 1.5; NEM=9.3 ± 1.5; URE=6.3 ± 0.7 fmol·ml^{-1}·mOsm^{-1}·kg H_2O^{-1}). The slopes of the relationships between plasma VP-RNP and Posm were markedly reduced in anesthetized animals compared to that observed for conscious animals (CON=2.54 ± 0.5; NEM=1.22 ± 0.18; URE=1.17 ± 0.24 fmol·ml^{-1}·mOsm^{-1}·kg H_2O^{-1}, $p <$ 0.0126). Our data suggest that anesthesia induced by either Nembutal or Urethane significantly reduces the responsiveness of VP neurons to acute salt loading, but the responsiveness of OT neurons is lowered by Urethane only.

438.14

CARBOCYANINE DYE LABELLED NEURONS AND PROCESSES OF *CARASSIUS AURATUS* PROJECTING TO THE PITUITARY. S.A. Johnston & J.N. Fryer. Dept. of Anatomy, University of Ottawa, Canada K1H 8M5.

Neurons projecting to the pituitary were retrogradely labelled following application of either diI or diO (D-282 or D-3883 Molecular Probes) crystals to either the pars distalis (anterior pituitary) or neurointermediate lobe (posterior pituitary) of goldfish perfused with 4% paraformaldehyde. The brain was sealed in a glass container for up to 20 wk at 23°C, in the dark. Vibratome sections (40 μm) were photographed through an epifluorescence microscope [rhodamine (diI) or fluorescein (diO) filter]. Cell groups were identified following Nissl staining of the sections. Labelled parvocellular neurons were found in the n preopticus periventricularis (NPP), n preopticus (NPO), superchiasmatic n (SC), n lateral tuberis pars anterioris (NLTa), posterioris (NLTp), and inferioris (NLTi), and between n recessus lateralis (NRL) and n recessus posterioris (NRP). Labelled magnocellular neurons were found in NPO, n lateral tuberis pars lateralis (NLTl) and its surrounding anterior hypothalamus (H); as well, a few scattered magnocellular neurons were labelled lateral to NRL. A major labelled tract arising from cells of the NPP, NPO, SC, NLTa, NLTl and the surrounding anterior H stretched in a semicircle along the lateroventral perimeter of the anterior H and entered the pituitary stalk. Fibers from NLTp, NLTi, and caudal H formed a caudobasal H tract projecting anteriorly to the pituitary stalk. Some CSF-contacting neurons were diI-labelled in NLTa and NLTp although the paraventricuar organ was not labelled. (Supported by MRC Canada)

438.15

DII-LABELLED NEURONS OF *CARASSIUS AURATUS* PROJECTING TO THE UROPHYSIS: DOUBLE LABELLED FOR UROTENSIN I OR II IMMUNOCYTOCHEMISTRY. J.N. Fryer & S. A. Johnston. Dept. of Anat., University of Ottawa, Canada K1H 8M5.

The caudal neurosecretory system of teleost fish and its neurohaemal organ, the urophysis, elaborate two unique neuropeptides urotensin I (UI) and urotensin II (UII). Spinal cord neurons projecting to the urophysis were retrogradely labelled following application of diI (Molecular Probes) crystals to the urophysis of goldfish perfused with 4% paraformaldehyde. The urophysis and attached caudal spinal cord (2-3cm) were sealed in a glass container for 20 wk at 23°C, in the dark. Vibratome sections (40 μm; horizontal plane) were free floated and colabelled for either UI or UII for 48 hr in the dark (FITC) and photographed (rhodamine or fluorescein filter; Elberger'89). Cell groups were identified following Nissl staining of the sections. All the diI-labelled cell populations occurred within 3 mm from the urophysis. The more caudal diI-labelled cells varied in size and were in dense clusters. The more rostral cells were magnocellular (30 μm) and diffusely distributed. Some caudal spinal cord neurons were not labelled with diI. Rostral to the urophysis, many somata, fiber and terminal distributions of diI, UI-ir or UII-ir overlapped, but a portion of UI-ir neurons were not urophysiotropic and showed many distinct plexi terminating on diI-labelled cell clusters. Via these terminations UI may also modulate the action of other urophysiotropic (UII?) neurons. Some UI-ir fibers also appeared to project anteriorly in the spinal cord. In conclusion, many caudal spinal cord cells project to the urophysis; a large population of these have been confirmed to be UI or UII positive; and UI neurons may terminate locally within the caudal neurosecretory system or elsewhere within the CNS. (Supported by MRC Canada)

438.16

A BIMODAL RHYTHM OF OXYTOCIN IN THE ANTERIOR PITUITARY GLAND. B. J. Arey* and M. E. Freeman, Dept. Biol. Sci., Florida State University, Tallahassee, FL 32306

We have recently proposed that oxytocin (OT) is the neurohormone responsible for the endogenous stimulatory rhythm (ESR) regulating prolactin (PRL) secretion (Arey and Freeman, *Endocrinology*, 124:878, 1989). The ESR is a bimodal rhythm with a nocturnal (N) component that peaks at 0300 h and a diurnal (D) component that peaks at 1700 h. In this study, we have investigated the concentration of OT in the anterior (AP) and posterior (PP) pituitary as well as in the peripheral circulation in order to determine if OT is responsible for stimulating PRL secretion during the periods of the ESR. Ovariectomized rats were decapitated every 2 h over a 24 h period. Each AP or PP was extracted with 2 N acetic acid. OT was measured in extracts and serum by RIA. Extracts from both the AP and PP caused displacement which was parallel with OT standards in the OT RIA. The AP contained a bimodal rhythm of OT concentration. Low levels of OT were present at 1200 h and 2200 h. A N surge began at 2400 h and peaked by 0200 h. OT concentrations at 0200 h were 6-fold greater than at 1200 h ($P<0.01$). Another, smaller D surge of OT in the AP began at 1700 h and peaked by 2000 h. The OT concentration of the AP at 2000 h was 3-fold greater than at 1200 h ($P<0.05$). The concentration of OT in the AP then declined to baseline by 2200 h. There was no detectable diurnal rhythm of OT in either the PP or peripheral circulation. Taken together, these data suggest that OT is secreted into the AP in a bimodal rhythmic fashion with a periodicity coincident with the periodicity of the ESR for PRL secretion. Furthermore, it provides further evidence for a role for OT as the neurohormone responsible for the ESR. Supported by NIH, HD-11669.

438.17

PCR ANALYSIS OF RAT PINEAL TISSUE FAILED TO DETECT OXYTOCIN OR VASOPRESSIN mRNA. M.M. Prechel*, M.R. Kelley* and W.H. Simmons. Loyola Univ. Med. Center, Maywood, IL 60153

Three neurohypophyseal peptide hormones have been detected in mammalian pineal glands. Two are oxytocin (OT) and vasopressin (AVP); the third is similar to arginine vasotocin (AVT) but has not yet been satisfactorily identified. These peptides are synthesized together with their carrier neurophysins, from single precursor mRNAs. The site of origin of the peptides found in the pineal is not known. The present study utilized the polymerase chain reaction (PCR) to look for evidence of neurohypophyeal peptide synthesis within the rat pineal gland. The PCR primers were complimentary to highly conserved regions of the hormone-neurophysin precursors, common to the OT, AVP and AVT messages, and bracketed a region of 239 base pairs (bp) of cDNA sequence. The PCR templates were cDNA obtained by reverse transcription of RNA from 19 day rat hypothalami and pineal glands, and from toad (B. marinus) brain. All three PCR products showed an ethidium bromide stained band at the 240 bp region on a 1.5% agarose minigel. But Southern blot analysis showed that only the rat hypothalamic cDNA hybridized with internal oligonucleotides specific for OT and AVP. These results indicate that the neurohypophyseal cDNA found in pineal does not represent message for OT or AVP, and suggest that these hormones are not synthesized locally. Furthermore, sequencing the novel pineal cDNA may soon establish the identity of the third, unknown pineal hormone. (Support: NSF and LUMC Potts Awards).

HYPOTHALAMIC-PITUITARY-ADRENAL REGULATION: STEROIDS

439.1

OVARIECTOMY (OVX) AND ESTROGEN (E) REPLACEMENT IN FEMALE RATS: EFFECTS ON ACTH, CORTICOSTERONE (CORT), MINERALOCORTICOID RECEPTOR (MR), AND MR mRNA. L.H. Burgess and R.J. Handa. Dept. of Cell Bio., Neurobio. and Anat., Loyola Univ., Stritch Sch. of Med., Maywood, IL 60153.

The E status of the female rat affects its endocrine response to stress. To explore this effect, we measured plasma ACTH and CORT, MR, and MR mRNA in 21 day OVX and OVX rats given E for 21 days. Pre- and post-stress CORT levels were increased ($p<0.05$) in E treated rats. Serial blood sampling revealed a time by E treatment interaction ($p<0.04$) following footshock. Both ACTH and CORT were greater ($p<0.05$) at 60 min.in E treated rats. This suggested an impairment of the CORT negative feedback mechanism. We therefore measured glucocorticoid receptor and MR levels in various brain regions with an in vitro binding assay using ^{3}H-CORT, RU 28362, and dexamethasone. E treated rats showed elevated ($p<0.05$) levels of MR in the hippocampus (HIPP) and hypothalamus. Preoptic area (POA) and HIPP MR mRNA levels were measured with an RNase protection assay using a 180 bp cRNA transcribed from $prMR_{EH}$. In OVX rats there were 4.6±0.5 fmol MR mRNA/mg RNA in HIPP and 1.0±0.1 fmol/mg RNA in POA. E treatment for 1 day lowered MR mRNA levels to 3.8 ± 0.5 fmol/mg RNA in HIPP and to 0.6±0.1 fmol/mg in POA. 21 days of E treatment lowered MR mRNA levels to 2.4 ± 0.5 fmol/mg RNA in HIPP. These data suggest that alterations in MR may underlie the changes in ACTH and CORT secretion seen in E treated rats.

439.2

THE AFFINITY OF THE MINERALOCORTICOID RECEPTOR IN HIPPOCAMPUS IS INDEPENDENT OF IN VIVO PROGESTERONE LEVELS. B.B. Turner, L.I. Holtsclaw*, and L. Xu*. James H. Quillen College of Medicine, East Tennessee State Univ., Johnson City, TN 37614.

The dissociation constant (Kd) of the mineralocorticoid receptor (MR) in the hippocampus and hypothalamus is several fold greater in female than in male rats. This study addresses the possibility that the observed affinity difference is due to the presence of progesterone in females. Twenty-four adult, female Sprague-Dawley rats were ovariectomized and injected s.c. for 10 days with either vehicle alone, estrogen (10μg), or estrogen plus either a low (50 μg) or high (500μg) dose of progesterone. Animals were adrenalectomized 20 hr before being killed under anesthesia by cardiac perfusion. Multiple blood samples were taken for determination of plasma progesterone. Binding parameters of soluble receptors have been measured in hippocampus and kidney slices of these animals by LH-20 gel exclusion chromatography.

Saturation plots of MR using 3H-dexamethasone in the presence of excess RU 28362 in kidney cytosols showed a significant difference in Kd between the vehicle control group and that receiving high dose progesterone (2.39 ± 0.40 nM vs. 6.08 ± 1.51 nM, $p < 0.05$). For kidney, the Kd for estrogen-treated group (2.43 ± 0.31 nM) was similar to control, and that for the low dose progesterone group (3.71 ± 0.45 nM) was intermediate between control and high dose progesterone. There were no differences in the Bmax of kidney MR among the four groups. Hippocampal binding showed no significant differences in either Kd or Bmax among the four groups. In hippocampus, the Kd of the control group was 1.34 ± 0.32 nM compared with 1.63 ± 0.43 nM in the high dose progesterone group. The Bmax of the control group in hippocampus was similar to that of the high dose progesterone group (121 ± 24 vs. 135± 18 fm/mg protein). The data indicate that the sex difference in the dissociation constant of the MR in hippocampus is not due to the action of progesterone in promoting dissociation of the MR-ligand complex. Supported by NIH 22158.

439.3

SEROTONIN (5-HT) REGULATION OF CORTICOSTEROID RECEPTOR BINDING IN CULTURED HIPPOCAMPAL CELLS: THE ROLE OF 5-HT-INDUCED INCREASES IN cAMP LEVELS. K. Betito, J.B. Mitchell, W. Rowe, P. Boksa, and M.J. Meaney. Douglas Hosp. Res. Ctr., Depts. of Psychiatry and Pharmacology, McGill Univ., Montréal, Québec, H4H 1R3, CANADA.

Recently we have shown that serotonin (5-HT) regulates the development of rat hippocampal type II corticosteroid receptors (CSR). We have reported that, in dispersed hippocampal cell cultures prepared from fetal rat (E19-20), 5-HT increased type II CSR binding (receptor binding measured using [3H]RU 28362). This effect required a minimum of 4 d exposure to 5-HT. The effect of 5-HT was mimicked by 5-HT2 receptor agonists and blocked by 5-HT2 receptor antagonists, such as ketanserin and mianserin. The present studies used this in vitro model to further explore 5-HT regulation of type II CSR binding. Cultures exposed to 10 nM 5-HT for 7 d showed a significant ($p<.01$) increase in type II CSR binding that persisted for at least 30 days following the removal of 5-HT, thus mimicking the long-term developmental effects seen in vivo. Cultures treated with 10 nM 5-HT showed a significant elevation of cAMP levels (~400%) that persisted for 7 d following 5-HT treatment. The effect was blocked by 100 nM ketanserin, and mimicked by 5-HT2 receptor agonists. Treatment of cultured hippocampal cells with the cAMP analog, 8-bromo cAMP, for 4 d produced a dose-related increase in type II CSR binding. The effect of 8-bromo cAMP was maximal at 10 μM concentrations, and the magnitude of the effect was comparable to that seen for 5-HT (160% vs 188%). Preliminary data indicate that activation of endogenous cAMP by forskolin treatment also increases type II CSR binding. Taken together with our previous findings, these results suggest that 5-HT is involved in the development of type II CSR binding and that the effect of 5-HT may be mediated by 5-HT-induced increases in cAMP levels.

439.4

THE ROLE OF BRAIN MINERALOCORTICOID AND GLUCOCORTICOID RECEPTORS ON CRF INDUCED HORMONAL AND CARDIOVASCULAR RESPONSES. S.M. Korte*, G.A.H. Bouws* and B. Bohus*. (SPON: European Brain and Behaviour Society) Department of Animal Physiology, University of Groningen, 9750 AA Haren, The Netherlands.

Behavioral and cardiac effects of centrally given CRF appeared to depend on adrenal hormones. Administration of the mineralocorticoid (RU 28318) and the glucocorticoid antagonist (RU 38486) 60 min before infusion of CRF were employed to investigate the role of the two receptor types on the CRF induced hormonal and cardiovascular changes. The antagonists were administered i.c.v. at a dose of 100ng/rat. CRF infusion itself (i.c.v., 300ng/rat) caused an elevation of plasma corticosterone (CS) and norepinephrine (NE) in combination with higher blood-pressure and heart rate. Prior treatment with the anti-glucocorticoid led to a matched enhancement of the NE response, and slight increase in the CS response. In addition, an increase in plasma epinephrine levels was seen, without an effect on blood pressure. The antiminer-alcorticoid did not have such effects. It is concluded that the brain glucocorticoid receptor regulated proces-ses lead to a very selective modulation of CRF-induced endocrine and physiological responses. This study was in part supported by the Foundation for Medical and Health Research MEDIGON (grant nr. 900-511-044).

439.5

IMMUNOCYTOCHEMICAL DEMONSTRATION OF MINERALOCORTICOID TYPE I RECEPTORS IN PRIMARY BRAIN CELL CULTURES. Y.-C. Chou*, Z. S. Krozowski*, W. G. Luttge and C. Sumners, Departments of Neuroscience and Physiology, University of Florida, Gainesville, FL 32610 and Medical Research Center, Prince Henry's Hospital, Melbourne, Australia 3004

It is now well accepted that mineralocorticoids, acting via specific receptors, have profound metabolic, neuroendocrine and behavioral effects on the brain. Previous studies from this lab have demonstrated [^{3}H]aldosterone binding to mineralocorticoid Type I receptors in astrocyte glial cultures similar to the binding found in brain and peripheral tissues. By using a polyclonal antiserum against a synthetic peptide (MINREC2) which corresponds to the hinge region of the human mineralocorticoid receptor, we have observed immunoreactive Type I receptors in a variety of brain cell cultures. MINREC2 antigenicity, detected by indirect alkaline phosphatase immunostaining, was found in both nuclei and cytoplasm of cells in neuronal cultures (containing 90% neurons), astrocyte glial cultures and "mixed" cultures (containing 50% neurons/50% astrocytes). Both neurons and astrocyte glia displayed immunoreactive Type I receptors. Because different types of cultures were grown using different sera, e.g. plasma-derived horse serum (PDHS) (neurons) and fetal bovine serum (FBS) (astrocytes), it was important to examine the effects of these sera on the expression of Type I receptors in cultured cells. It appears that the immunostaining observed in neurons maintained in 10% PDHS is not different from that in 5% PDHS/5% FBS.

The presence of MINREC2 staining in cultured neurons and astrocyte glia suggests that in addition to the ligand binding properties, Type I receptors in these cells are also similar in structure, at least in the hinge region, with those seen in brain and peripheral tissues. (Supported by PHS grants NS-19441 and HL-36645).

439.6

SPLANCHNICOTOMY ALTERS ULTRADIAN RHYTHMS IN STEROID SECRETION BY THE RAT ADRENAL. M.S. Jasper and W.C. Engeland, Sect. of Neurobiology/ Dept. of Surg., Brown Univ./ RI Hospital, Providence, RI 02903.

An ultradian rhythm in corticosterone (B) secretion which is synchronized between animals has been demonstrated in the rat using intra-adrenal microdialysis (FASEB J. 4:A827,1990). To determine the effect of the splanchnic innervation of the adrenal on rhythmic secretion of B, intra-adrenal microdialysis was done after splanchnicotomy (SPX). Under pentobarbitol anesthesia, adult male rats were prepared with a jugular vein catheter and an adrenal probe constructed from cellulose fibers (9 kD MW cutoff). In SPX rats (n=6), the thoracic splanchnic nerve was cut proximal to the suprarenal ganglion. In sham rats (n=7), no nerve was cut. Experiments conducted 1 day post-surgery consisted of continuous collection of dialysate at 10 min intervals (flow rate=10 µl/min) from 1000 to 1800 hr, with blood sampling at 1030 and 1730 hr. Dialysate B and plasma B were measured by RIA. Plasma B was similar in both groups. Episodic secretion of B persisted after SPX. Time domain analysis using PC-Pulsar revealed no difference in pulse frequency, width, inter-pulse interval, or normalized pulse amplitude. To determine if underlying periodic components in B secretion might be altered by SPX, frequency spectra were calculated for individual rats by fourier analysis. Averaging individual spectra within groups revealed peaks at approximately 60 and 30 min in sham rats. In SPX rats, the 60 min peak remained, whereas the 30 min peak was attenuated; additional energy was observed between 90 and 220 min in SPX rats. Averaging secretory time-series before spectral analysis did not change the spectral profile, indicating that episodes are synchronized between animals in SPX and in sham rats. These data suggest that adrenal splanchnic innervation modulates ultradian rhythms of adrenal B secretion, but does not directly generate or synchronize these secretory episodes. Supported by NIH grant DK38951.

439.7

ROLE OF GTP BINDING PROTEINS IN ADRENALECTOMY-INDUCED CHANGES IN HIPPOCAMPAL CALMODULIN ADENYLATE CYCLASE ACTIVITY. M. N. Gannon, T. Akompong and B. S. McEwen. Laboratory of Neuroendocrinology, Rockefeller University, New York, NY 10021.

We have observed that adrenalectomy (ADX) attenuates calmodulin- (CaM) and forskolin- (F) stimulated adenylate cyclase (AC) activity in rat hippocampal (HC) membranes. This effect is prevented by high, but not low, corticosterone replacement in ADX rats. ADX has been reported to alter levels of specific GTP binding (G) proteins (Gsα and Giα) in rat cortex, an effect prevented by CORT replacement (Saito, N., et al., PNAS, 86:3906, 1989). We therefore investigated the role of G-proteins in ADX-effects on HC AC activity. In both sham and ADX HC membranes incubation with the G-protein antagonist, GDPβS, did not abolish ADX attenuation of CaM AC, but decreased CaM- more than basal- or F-AC activity. Substitution of Mn2+ for Mg2+ in the AC assay, which selectively uncouples G protein control of AC, abolished CaM stimulation, but ADX still attenuated F AC. ADX had no significant effect on HC membrane Gsα and Giα content, measured by Western blot (n=10). These results suggest that ADX effects on HC AC may not occur through modulation of G-protein(s) levels, despite the fact that HC CaM AC appears selectively dependent on G-protein(s). Supported by NIMH-MH-41256.

439.8

EFFECTS OF AGING ON THE HIPPOCAMPAL GLUCOCORTICOID AND MINERALOCORTICOID RECEPTORS. M. I. Morano and H. Akil. Mental Health Research Institute, The University of Michigan, MI 48109-0720.

The basal levels of plasma corticosterone do not differ between young and old animals. However, the recovery to basal corticosterone levels following the termination of restrain stress (1 h) is delayed in the aged rat. We have measured the cytosolic MR and GR binding capacity as well as the expression of the mRNAs of both receptors in the hippocampi of young (5 mo.) and old (26 mo.) F-344 rats. The binding capacities of both receptors (MR and GR) are decreased in the hippocampi of old rats (57 and 52 % of those in young rats, respectively). The removal of endogenous corticosterone by adrenalectomy (2 days) results in a significant increase of more of 100 % in the maximum binding of these receptors in both young and old animals. The mRNA contents of MR and GR are lower in old sham-operated rats than in the young ones ($p < 0.05$). Interestingly, following adrenalectomy, only the old animals showed up-regulation of the levels of these mRNAs reaching levels comparable to the young; however, the binding capacities of these receptors in old rats did not achieve the levels in young rats. These data point to a possible change in mRNA stability and translatability in aged animals or to an alteration of steroid receptor cell cycle with age. Supported by MH 42251, DA 02265 and Markey Foundation.

439.9

MODULATION OF HIPPOCAMPAL GLUCOCORTICOID RECEPTORS IN ADULT RATS BY ALTERED THYROID STATUS. E.O. Johnson, A.E. Calogero, D.R. Rabin, G. Cizza, P.W.Gold, G.P.Chrousos, and T.C. Kamilaris, CNB/NIMH & DEB/NICHD, Bethesda, MD, 20892.

Both glucocorticoid receptors (GR) Type I and II exist in various extrahypothalamic sites, in particular the hippocampus, and have been implicated in the basal and stress-associated negative feedback control of the hypothalamic-pituitary-adrenal (HPA) axis. We investigated the effects of altered thyroid status on the intracellular glucocorticoid type II receptor concentrations in the hippocampus of adult male SD rats with short- (7 days) or long-standing (60 days) hypothyroidism (thyroidectomy + placebo), euthyroidism (sham thyroidectomy + placebo) or hyperthyroidism (thyroidectomy + thyroxine, 50 ug/day). We measured hippocampal GR Kds and concentrations and plasma concentrations of IR-ACTH and IR-corticosterone (B). There were no significant differences in the apparent Kds for [3H]-DEX binding as a function of thyroid status or duration of treatment. Although hypothyroid rats exhibited no changes in intracellular GR concentrations, however, hyperthyroid rats had significantly lower concentrations of hippocampal cytosolic receptors after both 7 days and 60 days of thyroxine treatment ($p<0.001$). Plasma ACTH and B levels increased in hyperthyroid animals, suggesting increased HPA axis activity in these animals. These data indicate that i) thyroid hormones modulate hippocampal GR type II concentrations; and ii) increased HPA axis activity in hyperthyroid states can be explained by a lower number of GR and decreased glucocorticoid feedback inhibition.

439.10

MK-801 ANTAGONIZES METHAMPHETAMINE-INDUCED DECREASES IN NEURONAL CORTICOSTEROID RECEPTORS. M.T. Lowy, Dept. of Psychiatry, Case Western Reserve University Sch. of Med., Cleveland, OH 44106.

Previous work in this laboratory demonstrated that depletion of biogenic amines by reserpine decreases hippocampal type I and type II corticosteroid receptors (CR) (Lowy, 1990). The present study was designed to determine if methamphetamine (MA), a stimulant drug which is toxic to serotonin and dopamine neurons, also decreases neuronal levels of CR. Administration of MA (15 mg/kg) to 1 day adrenalectomized rats decreased hippocampal type I (-29%) and II (-15%) CR as well as striatal type II CR (-21%) measured 3 hrs later. Cortical and hypothalamic type II CR were not affected. Since previous studies have demonstrated that the NMDA antagonist, MK-801, can attenuate the neurotoxic effects of MA, the effect of MK-801 (2.5 mg/kg) on the MA-induced decrease in neuronal CR was examined. MK-801 antagonized the MA-induced decrease in hippocampal type I and II CR as well as striatal type II CR. To directly examine if excitatory amino acids can regulate hippocampal CR, kainic acid (10 mg/kg) was administered to 1 day adrenalectomized rats which were sacrificed 3 hr later. Kainic acid significantly decreased both hippocampal type I (-47%) and type II (-38%) CR. These results indicate that MA decreases neuronal CR via a NMDA sensitive mechanism and that excitatory amino acids may play a prominent role in hippocampal CR regulation.

440.1

PERIPHERAL NERVE INJURY CAUSES CUTANEOUS NOCICEPTORS TO BE EXCITED BY ACTIVATION OF CATECHOLAMINE RECEPTORS J. Sato* and E.R. Perl, Dept. of Physiol., Univ. of North Carolina, Chapel Hill, NC 27599

We have shown that after nerve injury but not in normal animals, sympathetic trunk stimulation (SS) or intraarterial norepinephrine administration (NE) causes a fraction of C-fiber polymodal nociceptors (CPM) to discharge or to show enhanced heat-evoked sensitization. To test whether these effects on nociceptor activity are mediated by peripheral catecholamine receptors, the great auricular nerve (GAN) of deeply-anesthetized domestic rabbits was partially damaged under sterile conditions and the animals allowed to recover for 10 to 24 days. Post-operatively, none of the animals exhibited signs of discomfort or skin alterations. Terminally, under deep anesthesia, responses of CPM units to SS, NE and stereotyped heat stimuli were recorded from GAN filaments central to the site of damage. The relatively specific α_2 antagonists, yohimbine or rauwolscine (0.3-1 mg/kg) reduced or blocked the CPM excitation by SS and NE for 40-180 min, and reduced CPM sensitization by heat to 60% of control values. These results suggest that sympathetic and NE excitation of nociceptors after nerve injury probably is mediated by α_2 membrane receptors, phenomena possibly related to the pathogenesis of some causalgic-like pain syndromes. (Supported by grants NS 10321 and 14899 from NINDS.)

440.2

CHRONIC INJURY TO AXONS IN THE INFRAORBITAL NERVE PRODUCES LITTLE SPONTANEOUS ECTOPIC DISCHARGE MICHAEL TAL and Marshall Devor Depts. Anatomy and Zoology, Hebrew University of Jerusalem, ISRAEL.

Do chronically injured trigeminal axons generate massive spontaneous discharge like many afferents in hindlimb somatic nerves ? In an initial operation all fascicles of the infraorbital nerve in 18 adult male rats were ligated tightly with 6-0 silk and severed just distal to the ligature. After 4-164 days the rats were reanesthetized and the damaged nerve was exposed for acute electro--physiological study. Axons recordings were made from microfilaments teased from the nerve 10-15 mm. proximal to the neuroma. Only 40 spontaneously active A-fibers were encountered in 462 microfilaments (0.086 fibers/ microfilament). For comparison, sciatic nerve neuromas 3-16 days postoperative averaged 0.87 spontaneously active units per microfilament (28 rats, 645 microfilaments). Thus,the incidence of ectopic activity in infraorbital neuromas was less than 10% that of sciatic neuromas. To confirm that the nerve had not undergone massive retrograde degeneration, single stimulus pulses were applied just proximal to the neuroma, counting the number of single units recruited as current strength was gradually increased. On average, microfilaments contained at least $\geq$3.2 myelinated axons. Based on this average, the 40 spontaneously active axons encountered represent $\leq$ 2.6% of the sample. The corresponding value for sciatic nerve neuromas was 13.1%.

440.3

EVIDENCE THAT FAST AXONAL TRANSPORT IS INVOLVED IN THE DEVELOPMENT OF MECHANOSENSITIVITY IN AN ACUTELY CUT NERVE. G.M. Koschorke*, R.A. Meyer, and J.N. Campbell. The Johns Hopkins Univ. School of Medicine, Baltimore, MD 21205

Severed nerves develop mechanosensitivity at their ligated ends within hours of injury. The rate of development of mechanosensitivity was temperature sensitive. Because axonal transport is similarly temperature sensitive, we postulated that axonal transport plays an important role in the development of ectopic excitability. To test this further and to determine if fast or slow axonal transport is involved, the sural nerve in anesthetized monkeys (macaca fasicularis) was tightly ligated at a proximal site to cut off the source of transported substances. At a site 85 mm distally, the nerve was tightly ligated and cut either 3 or 12 hours later. At 3 hr, but not at 12 hr, substances conveyed by fast transport would still be in the nerve between the ligation sites. Ten hours after the distal injury, action potential activity in A-fibers was recorded in response to a mechanical stimulus at the distal site. Only 3% of the A-fibers responded to mechanical stimulation for the 12 hr experiment, whereas 29% of A-fibers responded for the 3 hr experiment. These results are consistent with the hypothesis that the components required for mechanical-to-electrical transduction at sensory receptors are conveyed via fast axonal transport and accumulate at the nerve injury site to impart ectopic excitability. (Supported by NIH NS-14447 and DOD N00039-90-C-5301)

440.4

CHRONIC THORACIC DORSAL RHIZOTOMY ALTERS IMMUNOREACTIVE CGRP AND SUBSTANCE P IN THE DORSAL HORN OF GOATS. **H.E Sloan, V. Miletic, K.K. Bowen*, K.T. Foley* and G.S. Mitchell, Dept. Comp. Biosci. and Environ. Tox. Cntr., University of Wisconsin, Madison WI, 53706.**

Thoracic dorsal rhizotomy (TDR) causes pronounced ventilatory failure in goats during mild exercise, followed by rapid functional recovery over subsequent exercise trials (Mitchell et al., FASEB J. 2:A1508, 1988). As an initial approach to study spinal changes associated with functional deficits or recovery, we investigated the distribution of calcitonin gene-related peptide (CGRP; a marker for primary sensory afferents) and substance P (SP). Bilateral dorsal rhizotomies were performed from T2-T12 (n=3). Six to twelve months after surgery, the goats were sacrificed, and their spinal cords removed and immersion fixed. Transverse 50 μm spinal sections were processed for immunoreactivity using conventional PAP methodology. For comparison, sections from unoperated and sham-operated goats were processed similarly. At thoracic levels, CGRP fibers were concentrated in laminae I and II. Fiber bundles were also seen in the medial portion of lamina V with scattered CGRP fibers in the ventral horn. TDR nearly eliminated CGRP fibers in the dorsal horn; only a thin band of fibers that skirted the underside of the dorsal horn remained. At cervical levels, however, TDR goats showed increased CGRP labeling in laminae III and IV. There were no obvious differences in CGRP immunoreactivity in the ventral horn. SP immunoreactivity paralleled the CGRP pattern in both normal and TDR goats.

These results indicate that even 6 months following TDR, regrowth of CGRP containing afferents does not occur, and that functional recovery of ventilatory control does not result from their regrowth. However, plasticity in the spinal cord may have occurred and contributed to longterm compensatory mechanisms as suggested by increased CGRP and SP labeling at cervical levels - the spinal regions critically involved with the control of breathing. (NIH HL36780, HL01494, NS26850 and NIEHS Training Grant T32 ES-07015).

440.5

HISTOCHEMICAL EVIDENCE OF THE INVASION OF VENTRAL ROOT AFFERENT FIBERS INTO THE SPINAL CORD OF RATS SUBJECTED TO SCIATIC NERVE LESIONS DURING THE NEONATAL STAGE. K. Sheen, B.S. Chung, J.W. Leem and J.M. Chung. Marine Biomed. Inst., Depts. of Anat. & Neurosci. and of Physiol. & Biophys., Univ. Texas Med. Br., Galveston, TX, 77550.

Using a horseradish peroxidase (HRP) labeling technique, we examined the possibility that ventral root afferent fibers invade the spinal cord after a neonatal peripheral nerve lesion.

A total of 11 anesthetized adult Sprague-Dawely rats were used (7 neonatal sciatic neurectomized and 4 normal control rats). After the L4-L6 dorsal roots of both sides were cut, HRP (30%) was injected into the spinal cord. HRP labeled cells were counted in the L5 dorsal root ganglion (DRG).

In rats subjected to neonatal sciatic neurectomy, there was an average of 45 labeled cells in each of the L5 DRG on the experimental side, whereas the DRG on the contralateral side contained 11 labeled neurons. On the other hand, the L5 DRG of normal control animals had only an average of 1 labeled neuron. The average size of labeled cells (29μm) was significantly smaller than the size of random sampled DRG cells (34μm).

These results suggest that lesioning the sciatic nerve during the neonatal stage triggers sprouting of fibers of small DRG cells, and that these fibers invade the cord via the ventral root. (Supported by NIH grants NS21266 & NS11255 and a grant from Bristol-Myers Co.)

440.6

BEHAVIORAL EVIDENCE FOR THE DEVELOPMENT OF TRIGEMINAL NEUROPATHIC PAIN FOLLOWING LIGATION OF THE INFRAORBITAL NERVE IN THE RAT. B.P. Vos and R. Maciewicz. Pain Physiology Lab, Dept. of Neurology, Massaschusetts General Hospital, Charlestown, MA 02129.

Loosely ligating the rat's sciatic nerve produces behavioral changes consistent with neuropathic pain in the affected hind limb (Bennett & Xie, Pain, 1988). To develop an animal model of trigeminal neuropathic pain, we ligated the infraorbital nerve (ION) in a similar fashion in the rat, and studied behavioral changes related to facial somatosensory function.

Experimental rats received a unilateral ION ligation and a contralateral sham operation. In control rats a unilateral sham operation was done. We quantified face grooming behavior, thigmotactic scanning activity, and responses to mechanical stimulation (tap, vibrissae pull, von Frey hairs:1,2,4,9,15g, pin prick) applied to different areas of the face. Rats were tested prior to surgery and up to 60 days post operatively (PO).

A significant increase in ipsilateral face rubbing was observed in experimental rats between days 1-20 PO. From day 10 PO, a decrease in use of the ipsilateral vibrissae to explore the environment was also observed. From day 5 PO, innocuous and noxious mechanical stimulation of the ipsilateral ION dermatome frequently evoked stereotyped episodes of intense face rubbing in experimental rats (100% on day 25); this type of aversive response was never observed in control rats. The frequency of episodes of intense face rubbing in response to mechanical stimulation to the contralateral ION dermatome or outside the ipsilateral ION dermatome in ION ligated rats also increased over time and with higher stimulus intensities.

The behavioral changes suggest the presence of paresthesias or dysesthesias, mechanical allodynia and hyperalgesia in the facial area innnervated by the ligated ION. These results provide behavioral evidence for the development of trigeminal neuropathic pain following ligation of the ION in the rat.

440.7

CHANGES IN PCP BINDING SITES IN RAT SPINAL CORD IN A CHRONIC CONSTRICTION INJURY L. M. Aanonsen[1], S. I. Sloan[1], K. C. Kajander[2], G. J. Bennett[4] and V. S. Seybold[3] [1]Biology Dept., Macalester College, St. Paul, MN 55105; [2]Dept. of Oral Sciences and [3]Cell Biology & Neuroanat., University of Minnesota, Minneapolis, MN 55455; [4]NAB, NIDR, NIH, Bethesda, MD 20892

A chronic constriction injury of the sciatic nerve in rats has been proposed to represent a model of peripheral neuropathy (Bennett and Xie,1988). The injury, which is induced by tying loose ligatures around the sciatic nerve, results in hyperalgesic responses to chemical and thermal stimuli. Previous studies have revealed that there is modulation of opioid and SP binding sites in this model (Stevens, *et al.* and Aanonsen *et al.*, NS Abstr. 15: 103, 1989). In the present study, we examined changes in PCP receptor binding sites in laminae I/II in rat spinal cord.

Spinal segment L4 was obtained from control rats (N=5) and from rats 2, 5 and 10 days after nerve ligation (N=8 rats/group). Autoradiographic studies were performed on spinal cord sections using 5nM ^{3}H-TCP; nonspecific binding was determined by incubation of adjacent sections in the ^{3}H-TCP plus 30 µM PCP (Aanonsen and Seybold, 1989). Data were analyzed using computerized grain counting. Specific binding was determined by subtracting nonspecific binding from total binding in each area analyzed. Statistical analyses (one-way ANOVA with Dunnett's post-hoc test) revealed a significant change in ^{3}H-TCP grain density in laminae I/II on the side ipsilateral to the ligation, but not on the side contralateral to the ligation. A significant *increase* in ^{3}H-TCP grain density was observed on day 2 while a significant *decrease* was observed 10 days after nerve injury. The PCP binding site has been shown to reside within a cation channel and is linked to the glutamate-activated, NMDA binding site. Glutamate has also been shown to be released in the spinal cord in response to noxious stimuli. Thus, the apparent modulation of PCP binding sites in this study may reflect changes in release of glutamate from afferents during the 10 days following constriction injury. Supported by USPHS grants DA05309 & NS17702.

440.8

Changes in [^{125}I]-hCGRP Binding Sites in the Dorsal horn of Rat Spinal Cord following Dorsal Rhizotomy.
M. G. Garry and V.S. Seybold. Dept of Cell Biology and Neuroanatomy, Graduate Program in Neuroscience, University of Minnesota, Minneapolis, MN 55455.

Whereas receptor binding sites for many peptides have been described in the brain and spinal cord, there have been limited reports addressing the regulation of binding sites for neuropeptides (other than the opioids) within the central nervous system. Calcitonin gene-related peptide (CGRP) occurs in primary afferent neurons at the spinal level, although the role of CGRP in spinal processing is equivocal. In order to determine whether dorsal horn cells regulate their binding sites for CGRP, quantitative receptor autoradiography was used to measure the amount of [^{125}I]-human CGRP binding in the dorsal horn of the spinal cord at select times following dorsal rhizotomy. Multiple unilateral dorsal rhizotomies were performed (L1-S1). Binding was analyzed in spinal cord segment L4. There were no significant differences between the amount of CGRP binding within the right and left sides of the dorsal horn of untreated animals in any of the laminae examined. On the intact side of the experimental animals, low densities of binding sites were observed in the superficial laminae. Ipsilateral to peripheral deafferentation, however, significant increases in the amount of CGRP binding were observed at 4 and 8 days following rhizotomy. These changes were observed in the medial and lateral aspect of laminae I/II and in lamina V (3-way ANOVA, $p<0.05$). No changes were observed in lamina X.

These results indicate that CGRP binding sites are regulated by the extracellular concentration of CGRP. In addition, these data suggest that CGRP binding sites may normally be "down-regulated" due to basal levels of CGRP release.

440.9

ANALYSIS OF CGRP AND SP BINDING SITES IN RAT SPINAL CORD IN AN EXPERIMENTAL MODEL OF ACUTE, PERIPHERAL INFLAMMATION. M.T. Galeazza, C.L. Stucky, E.M. Jansen and V.S. Seybold. Graduate Program in Neuroscience and Dept. of Cell Biology and Neuroanatomy, University of Minnesota, Minneapolis, MN 55455.

The purpose of this study was to determine whether peripheral inflammation causes changes in the density of calcitonin gene-related peptide (CGRP) and substance P (SP) binding sites in the dorsal horn of the spinal cord, and the time course over which any changes may occur. Inflammation was induced by subcutaneous injection of complete Freund's adjuvant (75 µl, emulsified 1:1 with PBS) into the plantar surface of the left hindpaw. Animals were sacrificed 2 or 4 days after the injection. A group of uninjected animals served as a control. Before sacrifice, the thickness of each hindpaw was measured, and Evans Blue (50 mg/kg i.v.) was injected into the animal via cardiac puncture. After sacrifice, biopsy punches of skin were taken from each hindpaw for extraction and colorimetric determination of Evans Blue. The thickness of the inflammed paw, as well as the amount of extravasated Evans Blue in this paw were significantly increased at 2 and 4 days after injection of adjuvant compared to controls. [^{125}I]CGRP (0.1 nM) and [^{125}I]SP (0.05 nM) were used to label binding sites on tissue sections of spinal segment L4 from each animal. Incubation of adjacent tissue sections in radiolabeled peptide with 0.1 µM peptide was used to determine nonspecific binding. The density of autoradiographic grains within the emulsion overlying laminae I/II of each dorsal horn within each tissue section was quantified by computerized image processing. The amount of [^{125}I]CGRP bound in laminae I/II decreased ipsilateral to the inflammed paw at 4 days after injection of adjuvant. In contrast, the amount of [^{125}I]SP bound did not change within this region at the time points examined. These data indicate that the release of CGRP within laminae I/II of the dorsal spinal cord is increased in conjunction with acute inflammation. Studies funded by NS17702.

440.10

INCREASE OF SPINAL GLUCOSE UTILIZATION IN RATS WITH CHRONIC NEUROPATHIC PAIN AFTER SCIATIC NERVE LIGATION. J. Mao[1*], R.C. Coghill[1], A.F. Germano[4*], R. Cicciarello[4*], R.L. Hayes[2], D.D. Price[3] and D.J. Mayer[1]. Dept. of [1]Physiology, [2]Neurosurgery, [3]Anesthesiology, Medical College of Virginia, Richmond VA 23298; [4]Neurosurgical Clinic, University of Messina, Italy. Previous investigations in our laboratory have mapped spatial patterns of thermal nociceptive processing in the rat spinal cord. In the present experiment, we have sought to map CNS patterns of metabolic activity elicited by a model of neuropathic pain in unanesthetized rats by the fully quantitative [^{14}C]-2-deoxyglucose (2-DG) technique of Sokoloff *et al.*(1977). Peripheral neuropathy was produced by sciatic nerve ligation according to the method of Bennett *et al.*(1988). Ten days after nerve ligation, femoral vessels contralateral to the ligated limb were catherized for blood sampling and 2-DG administration. Rats were allowed to recover completely from forane anesthesia before 2-DG (50 µCi/rat) administration and the subsequent 45 min period of blood sampling. Upon the completion of blood sampling, rats were killed, and their spinal cords were removed, frozen, and sectioned at -20° C for autoradiography. Optic densities of autoradiographs were analyzed and converted to absolute rates of glucose utilization. Rats used in the 2-DG procedure had demonstrable hyperalgesia ipsilateral to ligation. These rats withdrew the ligated foot from radiant heat two to three sec earlier than the control foot, and their hindpaws on the ligated side were lifted in a guarded position. Analysis of variance indicated that there was a statistically significant increase of glucose utilization over basal levels ($p<0.05$) in the each of five sampled regions, laminae I-II, III-IV, V-VI, VII and VIII-IX of the spinal cord ipsilateral to the ligated side. The increase of glucose utilization occurred over spinal segments L3 to L5. The preliminary results suggest that neuropathic pain induced by sciatic nerve ligation results in a proloned and chronic increase in neuronal activity in spinal cord regions involved in nociceptive processing. Supported by Fidia Pharmaceuticals.

440.11

TOWARD SELECTIVE LESIONING OF MOUSE NOCICEPTIVE DORSAL ROOT GANGLION NEURONS BY CHROMOPHORE-TARGETED LASER PHOTOLYSIS. J.D. Macklis and L.C. Dang*. Department of Neurology, Program in Neuroscience, Harvard Medical School, The Children's Hospital, Boston, MA 02115.

Recent studies have demonstrated selective, noninvasive lesions to subpopulations of CNS neurons targeted for laser photolysis by the retrograde incorporation of nanoparticles carrying photoactive chromophores. Similar mechanisms are investigated here as a potential approach for selective lesioning of nociceptive neurons located in mouse dorsal root ganglia. Selective neuronal targeting, photolytic singlet oxygen production within labeled cells, and laser-tissue interaction were studied.

Mice were injected subcutaneously in one hindlimb with 250 nl of nanoparticles with incorporated chlorin e_6. Following survival times of 1 to 12 weeks, mice were perfused and evaluated histologically for the level and distribution of label within neurons of different diameters. Quantitative analysis of the intracellular chromophore concentration was performed using an *in vitro* model of neuronal labeling and a microassay for cytotoxic singlet oxygen production. Transmittance of 670 nm wavelength laser energy through paraspinal bone and soft tissue was measured directly and compared with the solution of an appropriate analytical model using the diffusion approximation equation.

Dorsal root ganglia ipsilateral to the injection sites displayed intraneuronal label predominantly within small diameter (10-25 um), "dark" neurons, thought to be primarily nociceptive with "free" peripheral axonal terminals, capable of incorporating the nanoparticles. Labeled cells were found to contain 100 to 1000 particles, approximately 1 mM chlorin e_6. Energy transmittance of 5%-20% was measured through 1-2 mm bone and tissue thickness. These results suggest that one joule of incident energy could effectively lesion 10^4 neurons. Supported by NS28279, HD00859, and the Alzheimer's Association.

440.12

SINGLE UNITS RECORDED FROM INTRALAMINAR THALAMIC NUCLEI IN PATIENTS WITH DEAFFERENTATION PAIN EXHIBIT HYPERACTIVITY. R. F. Young, P. C. Rinaldi, D. Albe-Fessard* and J. Chodakiewitz*. Div. of Neurosurgery, Univ. of California at Irvine Medical Center, Orange, CA 92668.

Animal and human studies suggest that pain related to deafferentation is accompanied by spontaneous neuronal hyperactivity in the dorsal horn of the spinal cord and ventral posterior thalamic nuclei. In patients suffering from chronic pain associated with deafferentation, electrical activity of single cells in intralaminar thalamic nuclei was recorded for the present study. These patients were undergoing stereotactic procedures under local anesthesia for implantation of chronic stimulating electrodes or for thalamotomy.

In 8 of 10 patients single units were identified which discharged in high frequency, often rhythmic, bursts. Interval histograms from computer analysis revealed these discharges to be generally of two types: short bursts comprised of 2-6 spikes with burst frequency of 1 to 4 per second and long trains of 30-80 spikes and similar frequency. Reconstruction of electrode trajectories from MRI and CT scans indicated that the cells recorded were located in regions corresponding to the lateral aspect of the mediodorsal, central lateral, central median and parafascicular thalamic nuclei. The activity of cells from these nuclei which are part of the intralaminar nuclear group suggests that in patients with pain related to deafferentation the hyperactivity of units appears to be more widespread in the CNS than previously appreciated. The results have implications for treatment of chronic pain and for basic understanding of pain mechanisms.

440.13

STREPTOZOTOCIN-DIABETIC ANIMALS DISPLAY A HYPERALGESIA OF NON-PROSTAGLANDIN ORIGIN

S.C. Ahlgren; Y.O. Taiwo, J.D. Levine*
Dept. of Anatomy, UCSF, Box 0452, San Francisco CA94143

The goal of this study was to use an animal model of diabetes mellitus in the rat to study changes in nociception consistent with those seen in human diabetics. Diabetes was induced in 200-300g male Sprague-Dawley rats with the specific pancreatic B-cell toxin, streptozotocin (STZ, 70 mg/kg i.p.). Hyperglycemia (blood glucose > 350 mg/dL) was detected by the second day. Mechanical nociceptive thresholds were measured on the dorsum of the hindpaw using a Randall-Sellito paw-withdrawal device (Ugo-Basile).

In the diabetic rats, a significant decrease in mechanical nociceptive thresholds was observed starting seven days after STZ ($p<0.05$, one-way ANOVA). This hyperalgesic state (approx 30% decrease in threshold) persisted for at least 10 weeks. The dose-dependence relationship for hyperalgesia induced by i.d. PGE_2, which is thought to act directly on nociceptors and bradykinin, which produces a sympathetic-dependent, PGE_2-mediated hyperalgesia were also unchanged. I.d. indomethacin did not affect nociceptive threshold. These studies suggest that hyperalgeisa in the diabetic rat is not due to alterations in the production or action of hyperalgesic prostaglandins. Current experiments are examining the contribution of nociceptior terminal second messenger systems to diabetic hyperalgesia. *(Supported by NIH grant NS21647)*

440.14

THE NUMBER OF SUBSTANCE P CONTAINING DORSAL ROOT GANGLION CELLS IS REDUCED AFTER LASER IRRADIATION OF THE RAT TIBIAL NERVE. U. Wesselmann and W. Z. Rymer, Dept. of Physiology, Northwestern University Medical School, Chicago, IL 60611.

Recent studies from our laboratory indicate that Q-switched Nd:YAG laser irradiation might have selective effects on properties of small sensory nerve fibers. Previously we have demonstrated that laser irradiation selectively impairs neural impulse propagation in small slow conducting afferent fibers (Wesselmann et al., Soc. Neurosci. Abstr. 14:698, 1988). In addition we have shown that the number of small DRG cells labeled with HRP is selectively reduced after laser irradiation of the rat tibial nerve, while the number of large sensory neurons is not affected (Wesselmann et al., Soc. Neurosci. Abstr. 15:102, 1989). Substance P (SP) is a neurotransmitter, that has been shown to occur preferentially in the cell bodies of small sensory neurons. In an attempt to further analyze the preferential effects of laser application on small sensory nerve fibers, we have studied the effects of laser irradiation (Q-switched Nd:YAG laser, pulse energy -70 mJ/pulse, irradiation time - 5 min) of the rat tibial nerve on the number of SP labelled sensory neurons in DRG L4 and L5. In control animals (n=4) the numbers of SP containing neurons were not significantly different between sides (left: 4404 ± 404, right: 4492 ± 397)*. In contrast, after previous laser irradiation of the peripheral nerve the number of SP containing DRG cells was significantly reduced on the laser-treated side (left: 2710 ± 327, right: 4460 ± 342, $P<0.001$)*. The functional significance of this finding remains to be examined electrophysiologically. Since a large number of SP containing neurons appear to have nociceptive functions, the selective effects of laser irradiation on this neuron class might lead to potentially useful applications for the treatment of chronic pain. (Supported by the Medical FEL Program ONR/SDIO N00014-86-K-0188) *: mean±SEM.

440.15

INCREASES IN GFAP IMMUNOSTAINING IN THE LUMBAR SPINAL CORD FOLLOWING SCIATIC NERVE INJURY. **C.Garrison, P.Dougherty, K.Kajander and S.M.Carlton. Univ. of TX Medical Branch & Marine Biomedical Institute, Galveston, TX 77550.**

Previous studies indicate that glial cells within the spinal cord respond to transection of a peripheral nerve. Although the signal for this response remains unknown, evidence supports a neurochemical mediation of this phenomenon. Neurochemical changes occur in the spinal cord gray matter in the animal model of experimental peripheral neuropathy (EPN). It is unknown if glial responses also result from this nerve insult. Therefore, in the following study, EPN was induced in one hindlimb of 3 rats; the contralateral limb received a sham operation. On post-surgical day 10, animals were perfused with 4% paraformaldehyde and L4-5 segments removed. The tissue was frozen sectioned (25μm) immunostained with glial fibrillary acidic protein (GFAP).

Counting revealed no significant difference in the number of GFAP immunostained cells in experimental versus control horns; however, measurements of grey matter density demonstrated a significant increase in staining density on the experimental side. In addition to further characterizing spinal cord changes in the EPN model, these findings confirm that astrocytes respond to peripheral nerve injury, possibly playing a role in nervous system pathology. Supported by NS11255, NS27910 and Bristol Myers-Squibb Corp.

SENSORY SYSTEMS—RETINA: RETINAL CHEMISTRY AND ANATOMY

441.1

SYNAPTIC ANALYSIS OF SEROTONERGIC NEURONS IN THE TURTLE RETINA.

W. D. Eldred and L. B. Hurd*.
Department of Biology, Boston University, Boston, MA 02215.

We used antiserum directed against serotonin to label select populations of bipolar and amacrine cells in the turtle retina (*Pseudemys scripta elegans*). The processes of labeled bipolar cells in the outer plexiform layer were commonly seen to make wide gap contacts with photoreceptors. Processes of serotonergic bipolar cells were rarely seen near photoreceptor ribbons. Labeled bipolar cells formed dyads onto either amacrine/amacrine cell or amacrine/ganglion cell pairs in stratum 1 of the inner plexiform layer. Outputs of bipolar cells in strata 4/5 were similar in type, but fewer in number than in stratum 1. Inputs to both labeled bipolar and amacrine cells in strata 1 and 4/5 were from unlabeled amacrine cells. Serotonergic amacrine cells made synapses onto unlabeled amacrine and ganglion cells in strata 1 and 4/5. Rare synaptic contacts from labeled amacrine cells onto unlabeled amacrine and ganglion cells were seen in the region of stratum 3. These results support the role of serotonergic bipolar cells in the OFF pathway of retinal processing, and provide data on the synaptic interactions of the serotonergic amacrine cells in the IPL. This research supported by EY04785 to WDE.

441.2

Localization Of Serotonin Uptake Mechanisms In Rabbit Retina. W.J.Brunken and A.Pis-Lopez* Dept. of Biology, Boston College, Boston, MA 02167

In mammalian retina, uptake mechanisms have been used as markers of indoleaminergic function. Previous kinetic analysis has demonstrated that two mechanisms with different affinities are present. Anatomically three classes of amacrine cells have been shown to take up 5HT. In this study, we have begun localize these transport mechanisms to anatomically identified cell classes.

Rabbit retina were exposed to exogenous 5HT in the presence or absence of zimelidine, an uptake blocker. In control experiments, anatomical demonstration of uptake at low doses of 5HT revealed a single plexus of fibers in the IPL and occasional amacrine cells (2.06 cells/mm^2); at higher doses of 5HT, the density of labelled cells increased (5.97 cells/mm^2) and the IPL staining was diffuse. Zimelidine was able to reduce by 25 to 50% the density of labelled cells depending on the concentration of exogenous 5HT. Together these data suggest that the low affinity and high affinity transport mechanisms may be localized in different cell classes.

Kinetic analysis of ^{3}H-5HT uptake supports this hypothesis. We have confirmed the existence of two transport mechanisms: one with high affinity (ca 10nM) and one with low affinity (ca 10 uM). Zimelidine reduced uptake by the low affinity transporter but did not affect the high affinity transport mechanism. Zimelidine also reduced the maximal uptake of 5HT by the retina at 45 and 60 minutes by 42 and 55% respectively.

Finally, we used ^{3}H-paroxetine to label the high affinity transporter. Scatchard plots revealed a single binding site with a B_{max} of 285 fmol/mg protein and a K_d of 0.35 nM. Zimelidine did not displace paroxetine binding suggesting that it is effective only at the low affinity transporter. These data suggest that only those cells labelled with low doses of exogenous 5HT or in the presence of zimelidine posses the high affinity 5HT transporter.

441.3

DOUBLE-LABEL ANALYSIS OF THE COEXISTENCE OF SOMATOSTATIN AND NEUROTENSIN IN AMACRINE CELLS OF THE CHICKEN RETINA. C.B. Watt, H.B. Li* and P.A. Glazebrook*. Alice R. McPherson Laboratory of Retina Research, The Center for Biotechnology, Baylor College of Medicine, The Woodlands, TX 77381.

A comparison of previous immunocytochemical studies reveals a striking similarity in the morphologies of the populations of somatostatinergic and neurotensinergic amacrine cells in the chicken retina. A double-label study was performed to determine if these two neuroactive peptides coexist in chicken amacrine cells. A routine double-label paradigm was performed on cryosections collected through each of four retinal quadrants (dorsolateral, dorsomedial, ventrolateral, ventromedial). Primary antibodies were raised in different species, while respective secondary antibodies were conjugated to either fluorescein- or rhodamine-isothiocyanate. Control experiments testing the specificity of primary and secondary antibodies indicated no cross-reactive tendencies. Importantly, an examination of more than eight thousand labelled cells in each of the four retinal quadrants revealed that all labelled cells express both somatostatin- and neurotensin-like immunoreactivity. Therefore, these results indicate the presence of a single population of chicken amacrine cells whose members contain both of these putative neuroactive peptides.

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

441.4

POSTNATAL DEVELOPMENT OF TYROSINE HYDROXYLASE-IMMUNOREACTIVE (TH-IR) NEURONS IN THE RABBIT RETINA. Giovanni Casini, Nicholas C. Brecha, Ellen S. Takahashi and Clyde W. Oyster, Depts. of Anatomy & Cell Biology and Medicine, UCLA and VAMC, Los Angeles, CA, and School of Optometry, UAB, Birmingham, AL.

Dopaminergic amacrine cells play important modulatory roles in retinal function. The present study examined the development of TH-IR neurons in the rabbit retina. Putative dopaminergic neurons were identified either in retinal sections or in whole mount preparations by standard immunohistochemical protocols using a monoclonal antibody to TH. The first TH-IR cells were observed in the inner nuclear layer between the 5th and the 7th postnatal days. These neurons displayed weak immunoreactivity, and from day 6 immature processes could be observed. In the following days, the intensity of immunoreactivity increased, and by day 12 most of the TH-IR amacrine cells showed one or two major primary processes which had secondary branches stratifying in lamina 1 of the inner plexiform layer (IPL). At day 21, TH-IR cell bodies approached the morphology and the staining intensity typical of the adult, and an almost continuous band of immunoreactivity was present in lamina 1 of IPL. Staining intensity in the IPL increased with further development, and by day 28 processes in laminae 3 and 5 were also encountered. These observations support earlier biochemical data and show that TH-IR neurons develop postnatally, reaching complete maturity after day 28.

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441.5

$GABA_A$ α AND β SUBUNIT IMMUNOREACTIVITIES IN THE RABBIT RETINA. Nicholas Brecha and Christine Weigmann*. Departments of Anatomy & Cell Biology and Medicine, CURE and Jules Stein Eye Institute, UCLA School of Medicine and VAMC-Wadsworth, Los Angeles, CA.

The existence of several $GABA_A$ subunits (α,β,γ and δ) and their differential distribution are consonant with the presence of multiple $GABA_A$ receptors. This study evaluates the cellular localization and distribution of two of these subunits, α and β, in the rabbit retina using immunohistochemical techniques. Retinas were fixed in 4% paraformaldehyde and sectioned perpendicularly to the vitreal surface with a cryostat or a vibratome. Monoclonal antibody bd-24, directed to the α subunit, labels processes distributed to laminae 1, 3 and 5 of the inner plexiform layer (IPL) and to the outer plexiform layer. Some amacrine and horizontal cell bodies are heavily labeled. Faint labeling is occasionally observed in some cells located in the ganglion cell layer. Monoclonal antibodies bd-17 and 62-3G1, directed to the β subunit, also label processes distributed to laminae 1, 3 and 5 of the IPL and a few amacrine cell bodies. No staining is observed in adjacent sections incubated in normal serum. The immunolabeling pattern observed in this study, along with earlier *in situ* hybridization studies, emphasizes the multiplicity and heterogeneity of $GABA_A$ receptor expression in the retina.

Bd-17 and bd-24 were generously supplied by Dr. J. G. Richards; 62-3G1 was generously supplied by Dr. A. L. de Blas.
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441.6

β-NERVE GROWTH FACTOR-RECEPTOR IMMUNOREACTIVITY IN THE DEVELOPING RABBIT RETINA. Dennis W. Rickman, Nicholas C. Brecha and David Dawbarn*, Depts. of Anatomy & Cell Biology, USC; Medicine and Anatomy & Cell Biology, UCLA, Los Angeles, CA; and Medicine, University of Bristol, U.K.

The effects of nerve growth factor (NGF) on the survival, neurite outgrowth and target innervation of developing neural crest-derived neurons are well-documented. Recent studies with radiolabeled NGF or antibodies to the NGF receptor (NGF-R) suggest that NGF may have similar effects on neuroectoderm-derived neurons in the developing CNS, including the retina. Here, we used a monoclonal antibody (ME20.4) to the human low-affinity β-NGF-R to examine the localization and temporal pattern of β-NGF-R expression in embryonic and early postnatal rabbit retina. At embryonic day 15 (E-15) ME20.4 labeled radially-oriented cells which extended from the ventricular edge to the inner limiting membrane. At E-29 staining was observed in Müller cells, some amacrine cell somata, numerous ganglion cell somata and axons in the ganglion cell axon layer (GAL). This staining pattern was observed throughout the early postnatal period (P-2 to P-10) with particularly heavy labeling of ganglion cell axons in the GAL. At P-21 no staining was detected in any retinal layers. These observations suggest that β-NGF-R is expressed transiently throughout the period of retinal differentiation and may play a role in the development of retinal lamination and target recognition by ganglion cell axons.

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441.7

Distribution patterns of parvalbumin immunoreactivity in the vertebrate retina

Pietro Paolo Sanna, Kent T. Keyser# Elena Battenberg, Harvey J. Karten# and Floyd E. Bloom
Department of Neuropharmacology, Research Institute of Scripps Clinic and #Department of Neuroscience, University of California at San Diego

Parvalbumin is a low molecular weight calcium binding protein which has been proposed to be involved in determining relaxation speed in muscle fibres and is also specific to discrete neuronal subpopulations. We have investigated the distribution of parvalbumin-like immunoreactivity (P-LI-IR) in the vertebrate retina. P-LI-IR was present in different classes of neurons with significant interspecies variation. The most consistent finding was that P-LI-IR was found within some but not all amacrine cells. In the goldfish retina only amacrine cells were labeled. In the chicken and pigeon retina P-LI-IR was restricted to two morphologically distinct subpopulations of amacrine cells. In the rat retina, a subset of amacrine cells and a subset of cells in the ganglion cell layer were labeled. On the basis of fluorogold retrogade tracing, the majority of the latter appeared to be ganglion cells. A similar pattern was found in the ground-squirrel. In the rabbit, horizontal cells showed P-LI-IR as described by others (Röhembeck J. et al., Neuroscience Letters, 77 (1987) 255-260), and a subpopulation of amacrine cells as well as some sparse cells in the ganglion cell layer were also immunoreactive. In the cat retina, the majority of ganglion and horizontal cells as well as a subset of amacrine cells were labelled (as reported by Röhembeck et al., *Ibid.*). In mammals, in partial confirmation of results described by others (Endo T. et al., Cell Tissue Res., 243 (1986) 213-217), horizontal and amacrine cells displayed P-LI-IR as did sparse cells in the ganglion cell layer.

441.8

CEREBELLAR PURKINJE CELL MARKERS ARE EXPRESSED IN RETINAL BIPOLAR NEURONS. A. S. Berrebi†, J. Oberdick, L. Sangameswaran, S. Christakos††, J. I. Morgan and E. Mugnaini†. †Lab. of Neuromorphology, U-154, Univ. of Connecticut, Storrs, CT. 06269 and Dept. of Neuroscience, Roche Institute of Molecular Biol., Nutley, NJ 07110 and ††Dept. of Biochemistry, New Jersey Med. School, Newark, NJ 07103.

Previous studies have been directed at the elucidation of neuron-specific gene expression in the mammalian central nervous system. We have identified a series of marker molecules that are expressed in cerebellar Purkinje cells with varying degrees of specificity. Here, we show by immunocytochemistry and Northern transfer and hybridization that two of these markers, namely L7 and PEP19, are expressed in the retina of mouse and rabbit, while a third marker, cerebellin, is absent. L7-like immunoreactivity (IR) is restricted to rod bipolar cells, while PEP19-like-IR is distributed in both rod and cone bipolars as well as in subsets of amacrine and ganglion cells. An antiserum to a fourth Purkinje cell marker, vitamin D-dependent calcium-binding protein-28kD (CaBP), reveals primarily axonless horizontal cells, but also subsets of rod bipolar, amacrine and, in the mouse but not in the rabbit, ganglion cells. The processes of immunoreactive cell bodies form discrete bands in the internal plexiform layer and cocktails of the antisera help distinguish their identity. Thus, the Purkinje cell markers can be used at the electron microscopic level to unravel the extremely complex neuropil of this retinal layer. Furthermore, knowledge of the retinal distribution of this panel of molecules is of general value for future studies of retinal neuronal typology, and can serve to map the densities of subsets of bipolar cells throughout the retina. The expression of L7 and PEP19 in bipolar cells and in Purkinje cells suggests a biochemical relationship between these two spatially distant neuronal populations.

441.9

GAP JUNCTION DISTRIBUTION IN CAT AND MONKEY RETINA VISUALIZED WITH MONOCLONAL ANTIBODY TO CONNEXIN32. N. Vardi, E. Hertzberg, and P. Sterling. University of Penna, Phila. PA 19104 and Albert Einstein College of Medicine, Bronx, NY 10461.

Gap junctions are known in mammalian retina from electron microscopy of freeze-fracture replicas and ultrathin sections. However, with neither ultrastructural technique is the overall pattern of gap junctions in the tissue revealed. Immunocytological localization of gap junctions at the level of the light microscope by PAP was therefore undertaken using a monoclonal antibody specific for connexin32, one of several gap junction proteins thus far identified. In the outerplexiform layer the stain formed a dark band; the profiles of photoreceptor terminals were revealed as rings by the even distribution of staining of their membranes. In the inner nuclear layer stain was absent except for dense, punctate deposits on horizontal cells and on certain somas in the bipolar and amacrine cell layers. In the inner plexiform layer stain was evenly distributed as fine granules. In the ganglion cell layer stain appeared as distinct granules within the cytoplasm of ganglion but not amacrine cells. The staining patterns in monkey and cat retina were similar. This distribution of connexin32 immunoreactivity is consistent with previous ultrastructural observations of gap junctions, but suggests the possibility of electrical coupling at sites where it has not previously been suspected (e.g. onto certain bipolar and amacrine somas). EY 00828 (PS); GM 30667 (EH)

441.10

SPONTANEOUS AND EVOKED RELEASE OF TAURINE FROM A P_2 SUBCELLULAR FRACTION OF THE RAT RETINA. J.B. LOMBARDINI, Texas Tech U. Hlth. Sci. Ctr. Lubbock, Texas 79430.

The role of taurine as a potential neurotransmitter in the retina has been questioned in numerous literature reports. One essential criterion for a neurotransmitter is release of the substance from presynaptic nerve terminals. The present studies were designed to investigate the ion requirements on the spontaneous release of taurine and the effects of depolarizing agents on the evoked release of taurine from a P_2 subcellular fraction of rat retina. The tissue preparation was loaded with [^{3}H]taurine under conditions of high-affinity uptake. The absence of either Ca^{2+} (+ 2 mM EGTA) or Cl^- (replaced by Br^-) from the superfusion medium had no effect on spontaneous release. However, replacement of Na^+ with choline caused a 60% reduction in the spontaneous efflux rate. Potassium (56 mM)-evoked release of taurine was unaffected by the absence of Ca^{2+} (+2 mM EGTA). Veratridine (100 μM) was not as effective as K^+ in stimulating [^{3}H]taurine release as determined by measuring the areas (in arbitrary units) under the release curves (K^+ = 14.4 units; veratridine = 1.19 units). Substitution of Li^+ for K^+ resulted in a much reduced evoked release of [^{3}H]taurine (Li^+ = 2.2 units). The data suggest that the high-affinity uptake of [^{3}H]taurine is into a pool of taurine that is non-vesicular. The results do not support the hypothesis that taurine is a neurotransmitter in the rat retina. (Supported by NIH grant EY04780).

441.11

IS ACETYLCHOLINE INVOLVED IN LATERAL INHIBITION IN THE *LIMULUS* LATERAL EYE? HISTOCHEMICAL DEMONSTRATION OF ACETYLCHOLINESTERASE IN THE LATERAL PLEXUS. Daniel L. Sambursky, and Steven C. Chamberlain, SUNY Health Science Center at Syracuse and Institute for Sensory Research, Syracuse University, Syracuse, NY 13210.

Standard histochemical procedures for visualizing acetylcholinesterase (AChE) produced positive staining on frozen sections of the lateral eye. Deletion of acetylthiocholine iodide or substitution of butyrylthiocholine iodide produced no staining. On the other hand, staining was not affected by the inhibition of butyrylcholinesterase (BuChE) with Iso-OMPA. These results suggest specific demonstration of AChE rather than false demonstration of BuChE.

Axons of both retinular and eccentric cells were stained between the base of each ommatidium and the optic nerve, although no staining was observed in the somata. In the lateral plexus, a large uniform population of fibers was stained. Stained fibers were restricted to the lateral plexus and vertical bundles of axons. A similar pattern of fibers exhibits histamine-like immunoreactivity (Battelle et al., *Invest. Ophthalmol. Vis. Sci.* Suppl. **30**:290), however the pattern of fibers with AChE staining differs from that of fibers with substance P-like immunoreactivity (Chamberlain and Engbretson, *J. Comp. Neurol.* **208**:304) or that of fibers containing octopamine (Evans et al., *J. Comp. Neurol.* **219**:369).

The AChE-stained lateral plexus fibers must be the collaterals of the eccentric cell axons that mediate lateral inhibition (Fahrenbach, *Proc. Royal Soc.* Lond. B **225**:219), and other studies support the status of ACh as a neurotransmitter in *Limulus*. Acetylcholine synthesis has been demonstrated in the lateral and median optic nerves and in the optic ganglia of the brain (Battelle, *Vision Res.* **20**:911); AChE has been partially characterized from *Limulus* ventral nerve cord (Townsel et al., *Comp. Biochem. Physiol.* **58C**:29); and both ACh and choline acetyltransferase activity have been demonstrated in synaptosomes from *Limulus* CNS (Newkirk et al., *Comp. Biochem. Physiol.* **70C**:177). Supported by NIH EY03446 and EY00667.

441.12

PROTEIN KINASE C-LIKE IMMUNOREACTIVITY IN THE DEVELOPING RAT RETINA. D. Zhang and H.H. Yeh, Dept. Neurobiology and Anatomy, Univ. Rochester Med. Ctr., 601 Elmwood Avenue, Rochester, NY 14642.

In the vertebrate retina, a monoclonal antibody against protein kinase C (PKC) has been shown to label preferentially rod bipolar cells. Here, we examined in the rat retina the developmental pattern and the dynamic changes of cells expressing PKC-like immunoreactivity (PKC-LI) throughout postnatal life until adulthood. This was motivated in part by the possibility that the antibody might be used as a selective marker for examining the development of rod bipolar cells in the rat retina.

Faint PKC-LI could first be detected on postnatal day(PD)-10, being limited to the central region of the retina and labeling cell bodies located at the scleral margin of the inner nuclear layer adjacent to the outer plexiform layer. On subsequent days, PKC-LI spread progressively toward the peripheral retina and axon terminal bulbs at the vitreal margin of the inner plexiform layer began showing the first signs of immunoreactive labeling. By eye opening (PD-15), PKC-LI in these cells intensified and their axons became immunoreactive. The axons traversed the entire vertical thickness of the IPL and divided into short branches before ending as terminal bulbs. By the end of the fourth postnatal week, the PKC-LI cells appeared mature, resembling bipolar cells found in the adult. The morphology and the location of PKC-LI cells in both the developing and adult retina are consistent with them being rod bipolar cells. Rearing rat pups in complete darkness starting from the day of birth resulted in a precocious expression of PKC-LI.

In summary, PKC is not expressed in immunohistochemically-detectable amounts until relatively late in retinal development and thus fails to be a useful marker for rod bipolar cells at the earliest stages of maturation. Our results indicate further that the timing and degree of PKC expression may be subject to regulation by light even before eye opening.

Supported by PHS grants NS24830 and NS01340

441.13

CLONING AND EXPRESSION OF A MOUSE RETINAL POTASSIUM CHANNEL. D. J. Klumpp*, D. B. Farbert, C. Bowest*, and L. H. Pinto. Department of Neurobiology and Physiology, Northwestern University, Evanston, IL 60208, and †School of Medicine, University of California at Los Angeles, Los Angeles, CA 90024.

We have screened mouse retinal cDNA libraries in an effort to characterize potassium channels of the mouse retina. Libraries were screened with oligonucleotide probes homologous to known potassium channels. We isolated cDNA clones spanning the complete coding region of MBK1, a putative potassium channel clone from mouse brain (Tempel *et al., (1988) Nature* **333**, 837). A cDNA containing the complete open reading frame was constructed by ligation of partial clones. mRNA transcribed *in vitro* was microinjected into *Xenopus laevis* oocytes. We measured membrane currents evoked by voltage clamp steps. A slowly activated outward current was evoked from a holding voltage of -90mV. This current had a maximum amplitude at about +40mV and was attenuated by TEA, 4-aminopyridine and Ba^{2+} but was not attenuated by Co^{2+} (1mM). The reversal voltage of the current increased as a function of increasing K^+. Thus, MBK1 encodes a potassium channel expressed in the retina. Supported by NIH.

441.14

OPSIN GENE EXPRESSION: CONTROL BY VITAMIN A IN RAT VS. FLY. W. Stark & M. Katz*[1]. Div. Biological Sci. & [1]Dept. of Ophthalmology, The Univ. of Missouri, Columbia, MO 65211.

In rat, vitamin A deprivation lowers rhodopsin content, as determined by spectrophotometric measurements; however, both EM immunocytochemistry and freeze fracture analysis indicate that opsin density in the outer segments is unaffected by vitamin A deficiency (Katz, et al., *Invest. Ophthal. Vis. Sci. Suppl.* **31**, 77, 1990). The rod outer segments decrease somewhat in size but otherwise have relatively normal morphology. The *Drosophila* syndrome differs: Deprivation (egg to adult) lowers sensitivity 100x (Stark et al. *Naturwissen.* **63**, 513-518, 1976) by reducing both rhodopsin (spectrophotometry) & opsin (freeze fracture) (Harris et al., *Nature* **266**, 648-650, 1977). Opsin & rhodopsin recover together with replacement (EM immunolabeling & microspectrophotometry) (Sapp et al. *Invest. Ophthal. Vis. Sci. Suppl.* **31**, 77, 1990); label is high in rough endoplasmic reticulum after 1 day. Vitamin A likely specifically regulates opsin synthesis via transcription, translation or post-translational modifications in fly but not rat. This is logical from respective photochemistry: fly's photointerconvertible rhodopsin 480 - metarhodopsin 580 system need only be synthesized once except for some daily turnover (Stark et al., *J. Neurocytol.* **17**, 499-509, 1988); rod rhodopsin bleaches into opsin & chromophore, the latter recycled through supportive retinal pigment epithelial cells & reapplied to the opsin already present.

441.15

SIGNALS THAT GENERATE THE CIRCADIAN RHYTHM IN THE ERG OF ANOLIS. C.J.Karwoski, A.P.Shaw*, C.R.Collazo*, X.Xu* & J.Xu.*. Dept. of Psychology, Univ. of Georgia, Athens, GA 30602.

The b- and d-waves of the electroretinogram (ERG) of the lizard Anolis carolinensis show a circadian rhythm (CR) in amplitude.

Removal of the parietal eye does not affect ERG amplitude or its CR. Also, the CR can still be phase-shifted by a new light-dark cycle.

Optic nerve section slightly decreases ERG amplitude and has complex effects on the ERG CR.

Removal of the pineal gland abolishes the ERG CR. Application of melatonin to the isolated eyecup reduces ERG amplitude.

Surgical controls (sham optic nerve section, sham pinealectomy, lesions to the forebrain and to the optic tectum) do not affect ERG amplitude or abolish the ERG CR.

An ERG CR is observed in some isolated eyecups.

These findings suggest that plasma levels of melatonin (which could be rendered non-periodic by pinealectomy) may carry circadian information to the retina. However, centrifugal signals in the optic nerve may also play a role, as might an oscillator within the eye.

Supported by NSF grant BNS-8616847 (C.Karwoski) and a Ford Foundation Pre-Doctoral Fellowship (C.Collazo).

441.16

LIGHT REGULATION OF MELATONIN SYNTHESIS IN PRIMARY CULTURES OF EMBRYONIC QUAIL RETINAL CELLS. M. E. Pierce and J. S. Takahashi. Department of Neurobiology and Physiology, Northwestern University, Evanston, IL 60208.

Melatonin plays a key role in the regulation of a number of rhythmic physiological processes in the retina. The development of vertebrate *in vitro* model systems to study retinal melatonin biosynthesis would greatly aid our ability to investigate the cellular mechanisms regulating rhythmic photoreceptor metabolism. We have been studying the regulation of melatonin biosynthesis in primary cultures of dispersed embryonic chicken and quail retinal cells. Cultures are prepared by enzymatically dispersing isolated retina that is free of retinal pigment epithelial cells. Cultures from both speices synthesize melatonin. However, in quail retinal cell cultures maintained on a 12 hour light : 12 hour dark cycle, melatonin is rhythmically synthesized with higher levels of melatonin being produced in darkness. In addition, preliminary experiments suggest that melatonin synthesis in quail retinal cell cultures may synchronize to a reversed light cycle. Melatonin synthesis in embryonic chick retinal cell cultures is not regulated by light, but can be modulated by treatments which affect levels of cAMP. We are currently studying whether quail cultures exhibit properties of a self-sustaining oscillator *in vitro*.

Supported by NEI #EY08467 (JST) and #EY06167 (MEP).

441.17

INTERACTIONS OF MELATONIN AND DOPAMINE IN THE RABBIT RETINA. Ethan D. Cohen and Robert F. Miller, Dept. Physiol. Univ. of Minn., Minneapolis, MN 55455

Melatonin has been reported to be a potent inhibitor of dopamine release in the rabbit retina (Dubocovich, '83). We examined the effects of melatonin, dopamine agonists, and dopamine antagonists on ganglion cells, inner retinal neurons, and the ERG in a superfused eyecup preparation. In the outer retina melatonin (5-50 μM) had little effect on cone dominated horizontal cells, while dopamine, and its agonists SKF38393 (D-1), and LY171555 (D-2) all caused a small depolarization of the horizontal cell resting potential. Curiously, dopamine antagonists SCH23390, had little effect on the horizontal cell light response, while metoclopramide (20-40 μM) caused only a slight increase in the horizontal cell light response. At the level of the inner retinal neurons the actions of dopamine and melatonin were antagonistic. Dopamine depressed the light response of many on-depolarizing units (including an identified AII amacrine), while melatonin increased them. Melatonin (5-25μM) increased the spontaneous firing rate of on-tonic ganglion cells, while dopamine depressed them. Melatonin decreased surround excitation in off-tonic ganglion cells, while it had little effect on the spontaneous activity of all on- or off- phasic ganglion cells studied. These studies support a role for melatonin inhibiting synaptic release by dopaminergic neurons. (Suppt'd by EY00844 and F32 EY06171)

441.18

LIGHT-MEDIATED REFLEXIVE CONTROL OF CHOROIDAL BLOOD FLOW IN THE PIGEON EYE. M.E.C. Fitzgerald and A. Reiner, Dept. Anat. & Neurobiol., UT-Memphis, Memphis, TN 38163.

A neural circuit has been identified in birds that may allow light to reflexively control choroidal blood flow in the eye. The components of this circuit are: retina-suprachiasmatic nucleus (SCN)-medial Edinger-Westphal (mEW)-ciliary ganglion-choroid (Reiner et al., TINS, 1983). We have previously used laser Doppler techniques (using a TSI LASERFLO® Monitor) to show that electrical activation of mEW (Reiner and Fitzgerald, ARVO, '89) or SCN (Reiner et al., ARVO, '90) yields increases in choroidal blood flow. In the present study we investigated whether this circuit mediates increases in choroidal blood flow in response to retinal illumination in pigeons.

Tissue overlying the superior pole of the eye was removed, the extraocular muscles curarized and the laser probe positioned against the sclera to measure choroidal blood flow. Periodic ten second illumination of the retina of this eye, with an AO fiber optic light 2 cm from the eye, consistently yielded increases (20-100%) in choroidal blood flow. Assessments of the occurrence of this light-mediated response in pigeons that had sustained complete unilateral destruction of mEW 3-4 weeks earlier indicated that destruction of mEW greatly attenuated this light-elicited increase in choroidal blood flow.

Thus, the SCN-mEW circuit may be a neural substrate by which increases in retinal illumination yield increases in choroidal blood flow. Effective control of blood flow by this neural circuit could play an important role in maintaining a constant environment for retinal photoreceptors during light exposure. Supported by EY-05298 (A.R.)

441.19

A BASAL LAMINA AND MÜLLER GLIA ENDFEET ARE MISSING IN AN INVERSELY LAMINATED TISSUE, REGENERATING FROM EMBRYONIC CHICKEN RETINAL CELLS.
P.G. Layer [1], E. Willbold [1]* and H. Wolburg[2]*. 1) Max-Planck-Institut für Entwicklungsbiologie, 2) Pathologisches Institut der Universität, D-7400 Tübingen, FRG.

Starting with dissociated embryonic chicken retinal cells of defined origin, two rotary culture systems can be generated that express areas consisting of complete sets of retinal cell layers (*in-vitro-retinae, IVRe*). When pigmented cells from the eye margin are included, the sequence of layers is identical with that of an *in situ*-retina (*outside-out IVRe*). In *IVRe* derived from dissociated retinal cells only, the order of layers is inversed (*inside-out IVRe*; ref. 1). Using ultrastructural and immunohistochemical methods, radial processes, photoreceptor inner segments and tight junctions are similarly observed in both systems. However, exclusively in *outside-out IVRe*, we find premature Müller glia endfeet connecting to a well-developed basal lamina that originates from the pigmented cell core. The fact that a basal lamina and glial endfeet are missing in *inside-out IVRe* strongly argues that these components contribute to tissue polarity during normal and pathological retinogenesis.

Ref. 1: Layer, P.G. and Willbold, E. (1989). *Cell Tissue Res.* 258: 233-242.

442.1

ADAPTATION IN BULLFROG SACCULAR HAIR CELLS INVOLVES AN ACTIVE MOTOR. J.A. Assad and D.P. Corey. Neuroscience Group, Howard Hughes Medical Institute; Dept. of Neurology, Massachusetts General Hospital; and Dept. of Neurobiology, Harvard Medical School, Boston, MA 02114

Bullfrog saccular hair cells adapt to maintained displacements of their stereociliary bundles in a manner suggesting a continuous adjustment of the tension applied to the transduction channels (Howard and Hudspeth, 1987; Hacohen *et al.*, 1989; *cf.* Crawford *et al.*, 1989). Calcium entry through transduction channels, which can be blocked by sufficient depolarization, appears to reduce the equilibrium tension (Assad, *et al.*, 1989). This model predicts that the bundle should pivot a small distance negatively when the cell is depolarized; such movements have been observed (Assad, *et al.*, 1989). Correlation of these movements with the adaptation would imply that an intracellular molecular motor underlies the adaptation process.

We have examined further the relationship of active bundle movements to adaptation. Movements were recorded in dissociated, whole-cell voltage clamped hair cells using video microscopy. In response to voltage steps from -80 to +80 mV, the bundles moved ~40 nm negatively with an exponential timecourse (tau = 150-250 ms). The return following repolarization to -80 mV was much faster, typically reaching baseline within 60 ms. This asymmetry in timecourse was also seen in the negative shift of the I(X) curve (reflecting increased tension on the transduction channels) in response to the voltage step. The steady-state voltage dependences of the movement and I(X) shift were also similar. Active bundle motion was only observed in cells that had appreciable transduction currents; moreover, the depolarization-induced movement and I(X) shift tended to wash out approximately in parallel. These results strengthen the hypothesis that adaptation is mediated at least in part by an active, tension-regulating motor.

442.2

SINGLE-STEP PURIFICATION OF HAIR BUNDLES AND PRELIMINARY CHARACTERIZATION OF CONSTITUENT PROTEINS. P. G. Gillespie and A. J. Hudspeth. Department of Cell Biology and Neuroscience, University of Texas Southwestern Medical Center, Dallas, TX 75235-9039.

Characterization of the molecular basis of mechanoelectrical transduction in hair cells has been hampered by difficulty in obtaining purified hair bundles, the organelles of transduction, and the necessity of detecting extraordinarily small amounts of protein. We have therefore devised an efficient, "twist-off" procedure for purification of hair bundles and have developed a highly sensitive, nonradioactive means of protein detection.

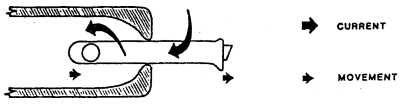
We isolate hair bundles by glueing the basal surface of a sacculus from the bullfrog (*Rana catesbeiana*) to the coverslip bottom of a cylindrical chamber. After embedding the organ in low-melting-point agarose, we rotate the solidified agarose cylinder with respect to the immobilized sacculus, shearing off the hair bundles at their relatively fragile bases. Light and transmission electron microscopy reveal few contaminants in the agarose plug containing the bundles. By the criteria of rhodamine-phalloidin labelling of F-actin, biotinylation of intracellular proteins, and exclusion of ruthenium red, over 90% of the stereocilia reseal after isolation. We visualize the hair-bundle proteins by silver staining or by biotinylation followed by chemiluminescence detection, which allows the detection on electroblots of as little as 500 fg (7 amol) of bovine serum albumin. Although actin constitutes over 75% of the protein in this preparation, or 10-20 ng per sacculus, at least 30 other proteins can be resolved by SDS-PAGE. Many of these proteins are exposed on the extracellular membrane surface, and several are extensively glycosylated; Triton X-114 extraction and phase partitioning suggests that some are integral membrane proteins.

442.3

OUTER HAIR CELL ELECTROMOTILITY: LOCALIZATION AND DISTRIBUTION OF THE FORCE-GENERATING MECHANISM. R. Hallworth, B.N. Evans*, and P. Dallos. Auditory Physiology Lab. (The Hugh Knowles Center) and Dept. of Neurobiology and Physiology, Northwestern Univ., Evanston, IL 60208.

The location and mechanism of outer hair cell electromotility are currently unresolved. A series of experiments was conducted to distinguish between cortical (membrane-associated) and intracellular locations. Isolated OHCs were partially drawn into a microchamber (Evans et al., 1990, in prep.), and voltage commands were applied across the cell.

Applied voltages resulted in antiphasic movements of the cell's ends, that is, while one half contracted, the other half extended. This result strongly implies that the mechanism is associated with the cellular cortex, since the potential gradients across the membrane halves are also in antiphase, but the potential gradient within the cell is unidirectional (see figure). When the percentage insertion, p, of the cells was varied, the displacement of either end could be described by a parabolic function, p(1-p). These observations suggest that the displacement is the cumulative sum of a large number of small motile elements driven in parallel. Measurements made at extreme percentage insertions deviated from the p(1-p) function, and were compatible with the assumption that the motile elements are restricted to the region between the cuticular plate and the cell nucleus.



(Supported by NIH grants DC00089, DC00708 and the Amer. Hear. Res. Found.)

442.4

MATURATION OF ELECTRICAL MEMBRANE PROPERTIES OF COCHLEAR HAIR CELLS IN THE EMBRYONIC CHICK. P.A. Fuchs and B.H.A. Sokolowski, Dept. Physiology, U. Colorado Med. School, Denver, CO 80262

The developing chick first responds to sound about embryonic day 11 (E11). Acoustic sensitivity and upper frequency limit increase with age, showing particularly marked improvement two days before hatching (E19). We have examined the electrical membrane properties of a topographically selected group of cochlear hair cells to determine what gated ionic currents might be acquired throughout this period. Hair cells were isolated from an area 0.8 to 1.2 mm from the apical tip of the adult cochlea, or from a proportionately located region of the smaller embryonic cochleas. Single hair cells were visualized with interference contrast optics on an inverted microscope and whole-cell, tight-seal recordings were made of membrane potential and transmembrane current.

Rapid, Ca-activated K current ($I_{K(Ca)}$) underlies electrical tuning at frequencies greater than 100 Hz in hair cells originating 1 mm from the apical tip of the mature cochlea. In contrast, hair cells from this cochlear region in the early embryo (E12-E14) had a much slower, voltage-dependent K current, (I_K) that in combination with Ca current produced slowly repetitive (at less than 20 Hz) action potentials. The Ca current was similar in kinetics and magnitude to that found in mature hair cells. One week later (E19) significant amounts of $I_{K(Ca)}$ were found in hair cells from this cochlear region. High frequency (140 Hz) voltage oscillations could be evoked in current clamp. The acquisition of Ca-activated K channels by hair cells may be essential for functional maturation of the chick's cochlea.

442.5

DIFFERENTIAL RESPONSE OF ISOLATED INNER AND OUTER HAIR CELLS TO STIMULATION BY POTASSIUM AND CALCIUM J. Schacht, D. Dulon* and G. Zajic* Kresge Hearing Research Institute, The University of Michigan, Ann Arbor MI 48109-0506.

Inner hair cells are generally considered the primary transducers in the mammalian cochlea while outer hair cells play a modulatory role in the transduction mechanism. Specifically, motile properties are thought to be unique characteristics enabling outer hair cells to modify basilar membrane micromechanics in response to efferent control. In isolated outer hair cells, increasing intracellular Ca^{++} leads to reversible cortical contractions and cell elongation by a mechanism dependent on calmodulin and structural proteins. Depolarization by K^+ triggers shape changes by an osmotic mechanism. A major question in determining the physiological correlates of such *in-vitro* responses is whether these phenomena are unique to outer hair cells.

Inner and outer hair cells were mechanically isolated from the guinea pig cochlea. Depolarization by KCl swelled both inner and outer hair cells by approximately 10% of their volume. The application of ionomycin, an ionophore permeabilizing the membrane to Ca^{++}, increased intracellular Ca^{++} levels in both inner and outer hair cells. This treatment, however, generated cortical contractions and elongation of outer hair cells only and did not affect the shape of inner hair cells. The results demonstrate that inner hair cells do not possess the mechanisms necessary for a contractile response to Ca^{++}. Calcium is thus a specific regulator of outer hair cell motility making this mechanism a most likely physiological modulator of a transduction feedback process. [Supported by NIH grant DC-00078]

442.6

EFFECTS OF OTOTOXIC LEVELS OF SALICYLATES ON SUBSURFACE CISTERNS OF OUTER HAIR CELLS. R. Dieler*, W.E. Shehata* and W.E. Brownell. The Center for Hearing Sciences, The Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205.

Cochlear outer hair cells (OHCs) possess force generating abilities. Their rapid electromotility may be structurally related to the subsurface cisterns (SSCs), a unique organelle composed of stacks of subplasmalemmal smooth parallel membranes. Ototoxic doses of salicylate have been shown to reduce otoacoustic emissions *in vivo* as well as OHC turgidity and electromotility *in vitro* (cf. Brownell,W.E., Ear & Hear., 11:82-92, 1990). In vivo studies (Douek,E.E. et al.,J. Laryngol. Otol., 97:793-799, 1983) have suggested that SSCs are an important intracellular target for salicylate ototoxicity.

We have superfused isolated OHCs from guinea pig cochleae with culture media containing sodium salicylate (2mM, 5mM, 10mM for 10 - 60 min.). Subsequently, the cells were fixed with an osmotically balanced glutaraldehyde solution and processed for transmission electron microscopy. OHCs maintained in standard culture media served as controls. The majority of control cells showed 1 - 13 well preserved, smooth, unfenestrated SSC layers. Supranuclear lamellar or Hensen bodies were only occasionally seen. Following salicylate exposure, the cisternal layers lost their parallel arrangement and became vesiculated forming fenestrated cisterns of random orientation . There was also a more frequent occurrence of irregularly located cytoplasmic membranous systems (probably Hensen bodies). The intermembranous cisternal distances appeared to be increased and in some instances disintegration of the outermost cisterns was seen. The present findings demonstrate a structural involvement of SSCs in the ototoxic response to salicylate and strengthen the possibility that the SSCs might be an important anatomical substrate for electromotility.

Supported by the Max Kade Foundation and Grants from ONR & NIH.

442.7

SHORT (OUTER) HAIR CELLS OF THE CHICK'S COCHLEA ARE HYPERPOLARIZED BY CARBACHOL. B.W. Murrow and P.A. Fuchs, Dept. Physiology, U. Colorado Med. School, Denver, CO. 80262.

Sensory receptor cells (hair cells) of the chick's cochlea are classified as short or tall by the ratio of their apical surface diameter to length of the cell body. In short hair cells this ratio is greater than 1. Short hair cells are situated over the basilar membrane, in a position analogous to that of outer hair cells in mammals. Further, it is thought that the efferent nerve supply to the cochlea ends preferentially on short hair cells, as on outer hair cells, and that these axons release acetylcholine to produce their inhibitory effect. We have examined the effect of the cholinergic agonist, carbachol, on hair cells isolated from selected regions of the chick's cochlea. Whole-cell, tight-seal recording was performed on cells that were exposed to a stream of 100 μM carbachol from a nearby perfusion array.

In short cells carbachol produced a large hyperpolarization (as much as 30 mV from a resting potential of -40 mV). In voltage clamp carbachol produced an outward current that could reach several hundred pA from a holding potential of -40 mV. Variation of the holding potential suggested that the current reversed near E_K. The carbachol response desensitized over the course of several seconds. Responses to carbachol were observed in 17 of 18 short cells. In contrast, carbachol had no effect on 18 tall cells from the same cochlear section, mimicking the proposed efferent innervation pattern.

442.8

VOLTAGE-GATED Ca^{2+} CURRENTS RECORDED *IN VITRO* FROM OUTER HAIR CELLS OF THE GUINEA PIG. X. LIN[1]*, R.I. HUME[2], A.L. NUTTALL[1], Kresge Hearing Research Institute[1] and the Dept. of Biology[2], The University of Michigan, Ann Arbor, MI 48109

Auditory efferent neural transmitter(s) may influence the transduction process in the organ of Corti by modulating voltage-gated ion channel(s) in the membrane of outer hair cells (OHCs). This hypothesis motivated our work on characterizing voltage-gated currents of the OHCs. Single isolated OHCs of the guinea pig were studied *in vitro* by whole cell voltage clamp techniques. OHCs were isolated enzymatically and mechanically from the apical two turns of guinea pig cochlea and maintained in short-term culture. The pipette internal solution contained (mM): KCl 130, EGTA 20, Hepes 10, Glucose 10, pH 7.4. Whole cell clamps were established in a modified Hank's solution (NaCl 130, KCl 4, $MgCl_2$ 2, Hepes 12.5, Glucose 10, pH 7.4). The external solution could be changed via a perfusion system. Currents (Leakage subtracted) in response to a series of depolarizing voltages from a holding potential of -110mV were recorded in the modified Hank's external solution. A transient inward current first appeared when cells were depolarized to about -70mV, but the responses to more positive test pulses were dominated by a delayed outward current with little inactivation. The outward current was completely suppressed by changing the medium in the recording chamber to a solution expected to suppress K^+ and Na^+ currents, which contained: KCl 4, $CaCl_2$ 4, TEA·Cl 100, 4-aminopyridine(4-AP) 10, TTX 100nM, Hepes 12.5 and Glucose 10. With this bathing solution, the inward currents recorded in response to the same test paradigm consisted of two components: (1) a fast-inactivating inward current first appeared at about -70mV, (2) a slow-inactivating steady inward current began around -30mV and peaked at about +20-30mV. The composition of the new external solution and the observation that the inward current didn't reverse for depolarizing potentials up to +80mV indicated that Ca^{2+} ions were the charge carrier. Similar inward currents could also be recorded when pipette internal solution was supplemented with 2mM TEA and 1mM 4-AP and cells were bathed in Hank's solution.

In conclusion, the data we have obtained so far support the idea that multiple types of voltage-gated Ca^{2+} channels as well as at least one class of K^+ channels are present in the membrane of the guinea pig OHCs.

(Supported by NS05785)

442.9

WHOLE-CELL CURRENTS IN MAMMALIAN VESTIBULAR HAIR CELLS. R.A. Eatock. Dept. of Physiology, University of Rochester, Rochester, NY 14642.

It is likely that the functional diversity of mammalian vestibular neurons in part originates in the hair cells; however, little direct information on the physiology of mammalian vestibular hair cells exists. We are examining the hair cells' membrane currents and voltages using the whole-cell GΩ seal technique. Hair cells from the utricles and semicircular canal organs of rats are mechanically dissociated following enzymatic treatment and are maintained in Hanks' Balanced Salt Solution. While both Type I and Type II cells can be identified, all of the data are from probable Type II cells. Depolarizing voltage steps typically elicit small inward currents followed by relatively large outward currents. These currents resemble the Ca and Ca-activated K currents in type II hair cells of other species. Depolarizing current steps evoke voltage responses that suggest heavily damped electrical resonances. The resonant frequency is voltage-dependent, varies among cells, and is correlated with the kinetics of the outward currents. (Supported by ONR)

442.10

IDENTIFICATION OF SPECIFIC CARBOHYDRATES IN THE CELL COAT OF THE TROUT SACCULAR EPITHELIUM. K.M.Khan[1], J.S.Hatfield[2]*, and D.G.Drescher[1]. [1]Laboratory of Bio-otology, Wayne State Univ. School of Medicine, Detroit, MI 48201, and [2]V.A.Medical Center, Allen Park, MI 48101.

A variety of possible functions have been proposed for the glycoconjugates present in the cell coat surrounding the stereocilia of the vertebrate inner ear. Glycoconjugates may hold the stereocilia together, prevent their fusion, and/or sequester cations essential for mechano-sensory transduction. We have reported the presence of a glycoconjugate-rich cell coat on the luminal surface of cells of the trout saccule (Assoc. Res. Otolaryngol. Abstr. 13: 361, 1990). The aim of the present study was to identify specific carbohydrate moieties of the cell coat of the trout saccular epithelium, using lectin probes. Trout saccules were dissected and fixed in Karnovsky's fixative (100 mM cacodylate buffer, pH 7.2) for 2 h. Specimens were treated with biotinylated lectins, labeled with HRP-conjugated avidin, post-fixed in buffered 1% OsO_4, dehydrated in ethanol, and embedded in LX-112 resin (Ladd Res. Industries). As controls, specific inhibitory sugars (final concentration, 0.2 M) were added to the lectin solutions. Glucose, galactose, fucose, mannose, *N*-acetylglucosamine, and *N*-acetylgalactosamine were found to be present on the luminal surface of the sensory and non-sensory cells. However, variations in the intensity of staining, associated with the sensory and the non-sensory cells, suggested biochemical heterogeneity and quantitative differences among various oligosaccharide chains present in the cell coat. In control specimens, staining was either eliminated or reduced. The presence of these carbohydrates in the cell coat of the trout saccular epithelium is consistent with the distribution of carbohydrate moieties present in the mammalian inner ear structures (Gil-Loyzaga et al., Hear. Res. 20: 1-8, 1985; 34: 149-156, 1988). These results suggest biochemical similarities between the cell coats surrounding teleost and mammalian inner ear structures.

442.11

POSTNATAL REMODELING OF COCHLEAR NEURON ARBORS IN A MAMMALIAN EAR. Stephen M. Echteler. Auditory Physiology Lab. and Dept. of Neurobiol. and Physiol., Northwestern University, Evanston, IL 60208.

In adult mammals, inner (IHC) and outer (OHC) cochlear hair cells are contacted by afferent neurons which are morphologically distinct and completely segregated. iHC afferents are thick myelinated neurites which project radially within the cochlear duct, produce few branches and typically innervate the nearest one or two receptors with punctate bouton endings. OHC afferents are thin unmyelinated fibers which extend for up to 600 μm before branching serially to innervate from 5 to 50 receptors.

To determine how these two strikingly different patterns of hair cell innervation are constructed I have examined early postnatal changes in the arborization of individual cochlear neurons in the Mongolian gerbil. Neurons were labeled through injections of Horseradish peroxidase into the apical turn of freshly excised cochleae which were subsequently maintained in vitro for three to four hours.

During the first few postnatal days (P0 to P2) some neurons had peripheral neurites which did not yet reached the IHC zone. These neurites were capped with growth cones of varied morphology depending upon their proximity to their hair cell targets. Other neurons had more complex arbors with neurites contacting both IHCs and OHCs. During the next two postnatal days (P3 and P4) neurons contacting both receptor types became increasingly rare. By P5, individual cochlear neurons contacted either IHCs or OHCs. At each postnatal stage the form displayed by an immature cochlear neuron depended not only upon its age but also its position within the cochlear spiral; neurons located more apically generally possessed peripheral arbors which were less well developed. (Supported by NIDCD grant R29 DC00493).

442.12

BASAL/APICAL DIFFERENCES IN THE EFFECTS OF COOLING ON THE RESPONSES OF SINGLE COCHLEAR NERVE FIBERS IN THE GERBIL. K.K. Ohlemiller and J.H. Siegel. Auditory Physiology Lab. (Audiology) and Dept. of Neurobiology and Physiology, Northwestern Univ., Evanston IL 60208.

We have developed a method for rapidly cooling and rewarming the gerbil cochlea while recording from auditory nerve fibers. Single fiber responses are examined under conditions where cochlear temperature is either 35-39°C or 29-32°. Using this technique we are able to elicit stable and reversible changes in single fiber spontaneous discharge rates (SR), as well as tuning and rate-intensity curves. Cooling-related changes in frequency tuning depend on characteristic frequency (CF). Fibers with CFs below and above 8 kHz exhibit increases in CF threshold of about 0-10 dB and 10-20 dB, respectively. This pattern is similar to stimulus frequency-dependent increases in compound action potential thresholds observed during passive cooling of the cochlea to 30-31°. Tip/tail ratios of tuning curves are reduced. Sharpness of tuning (as assessed using Q_{10dB}) and CF are, on average, unchanged. We recently reported that the distributions of SR, saturation firing rate (R_{sat}) and intensity curve slope in gerbil are different for fibers with CFs below and above 3-4 kHz (Ohlemiller, Echteler, and Siegel, submitted). Those results held for cochleas near 37°. When the relative changes in each of these characteristics during cooling are examined for fibers with CFs below and above 3 kHz, we find the following: R_{sat} is reduced by 10-50%, regardless of CF. SR is reduced by about 10% in low-CF fibers and about 80% in high-CF fibers. This difference is significant (t-test, $p < .001$). Intensity curve slope is also differentially affected in the two groups. The slopes of intensity curves of low-CF fibers are reduced (sign test of matched pairs, $p < .0002$) by 30-80%. For high-CF fibers no significant change in slope is observed ($p > .4$).

(Supported by R01 DC00350 to JHS and NRSA MH09565 to KKO)

442.13

VOLTAGE-ACTIVATED MEMBRANE CURRENTS DIFFER BETWEEN MAMMALIAN TYPE I AND TYPE II HAIR CELLS. K.J.Rennie and J.F.Ashmore. Dept. Physiology, Med. Sch., Bristol BS8 1TD, U.K.

The mammalian vestibular system contains two types of hair cell (designated I for the flask shaped and II for the cylindrical shaped) which differ in their morphology and synaptic connectivity. We have isolated both types from the guinea pig crista ampullares to study the ionic differences in membrane currents. It is convenient to further divide type II cells into tall (IIt) and short (IIs) sub-types. These have distinguishable ionic properties.

Using tight-seal whole-cell recording (pipette containing KF/KCl + BAPTA), the mean zero current potential was -49.5±9.2mV (n=57), with no significant difference between cell classes. All cells showed a prominent outward K-current which activated above -45mV. 62% of type IIt, 31% of type IIs and 17% of type I cells showed a current classified as an A current. A further component of the outward current was invariably reduced by 10mM or 30mM TEA (n=11), or by 200μM Cd (n=8), indicating that all cell types have a K(Ca) current. Cs-containing pipettes greatly reduced outward currents in type IIt cells, but left a small (300pA) inward transient which was blocked by 240μM Cd. In type IIs and type I cells, the outward current was not reduced by internal Cs, suggesting an underlying K channel with a different selectivity. At holding potentials more negative than -90mV, type I cells showed outward rectification, eliminated by more positive holding potentials, presumably due to Ca loading. Such elevated intracellular levels of Ca activated a conductance with properties of a non-selective cation channel.

The results indicate significant differences between cell types. In particular the regulation of type I cell currents by internal Ca may determine the dynamic properties of the cell's response during labyrinth rotations.

Supported by the Wellcome Trust

SOMATOSENSORY CORTEX AND THALAMOCORTICAL RELATIONSHIPS III

443.1

LIMIT OF ACCURACY OF MEG DIPOLE LOCALIZATION ON THE HUMAN SOMATOSENSORY CORTEX REVEALED BY MAPPING MAGNETIC (MEG) RECORDINGS ONTO THREE-DIMENSIONAL MRI RECONSTRUCTIONS. U. Ribary, J. Suk*, J. Cappell*, F. Lado, A. Mogilner and R. Llinás. Dept. of Physiology & Biophysics, New York University Medical Center, New York, NY 10016 U.S.A.

A 14 channel neuromagnetic measuring system (BTi) was used to analyze the magnetic fields over the left hemisphere of three normal male subjects (18-40 years old) in order to locate the magnetic response sources to contralateral tactile stimulation of the thumb, the index and the little finger. The stimuli were randomly provided by three Piezo buzzers vibrating at a frequency of 250Hz and triggered at 100 msec, after the start of each recording window of 300 msec, for a duration of 100 msec and an interstimulus interval of 400 to 600 msec. The orientation of the subject's heads in relation to the MEG recording probes was calculated using a Probe Position Indicator (PPI) and a head-based coordinate system (Yamamoto et al., 88' Proc. Natl. Acad. Sci.). The dipole locations were based on the main peak around 60 msec after stimulus onset, demonstrating a spatial magnetic field reversal over the hemisphere. The active dipole sources determined were then projected onto the Magnetic Resonance Image (MRI) of the individual subjects providing anatomical localization. Each measurement was repeated 8 times over 3 months and demonstrated an accuracy ranging from ± 0.46 mm to ± 2.28 mm (SEM). The MRI slices were also used to construct a 3-dimensional image for a better visualization of the spatial distribution of the calculated sources. All sources from the three fingers were found to be located on the postcentral gyrus (in SI) and distinct from one another. There was a most significant difference in source location between the little finger and the thumb with the former being more superior than the sources of the other two fingers. The response amplitudes also showed significant and consistent differences with the thumb having the largest, the index finger the second largest and the little finger the smallest response. The high level of statistical significance of these measurements indicate that the MEG technique has allowed precise and fast determinations of brain function in a non-invasive environment.

443.2

REGIONAL CEREBRAL BLOOD FLOW CHANGES FOLLOWING NOXIOUS THERMAL STIMULATION USING SUPERPOSITION OF SPECT AND MRI. A.V. Apkarian, N.M. Szeverenyi*, S.H. Manglos*, R.T. Stevens and F.D. Thomas*. Depts. of Neurosurg. and Radiol.*, SUNY Hlth. Sci. Center, Syracuse, NY 13210.

Single photon computed tomography (SPECT) is used with various brain perfusion radiopharmaceuticals to monitor the metabolic activity of the brain non-invasively. Increased metabolic activity can be secondary to increased neural activity which renders SPECT useful in monitoring brain activity during sensory stimulation. In this study SPECT images were generated in humans and anesthetized baboons when the hand was immersed in a noxious water bath (46-50 ^{o}C) and compared to the hand immersed in neutral temperature water. Technetium-99m-HMPAO was injected iv during stimulation, the head placed in a special head holder and the brain scanned with a 3 headed rotating SPECT scanner (TRIAD). SPECT and stimulus-control SPECT images were suprimposed on congruent high resolution magnetic resonance images (MRI). The head holders supported fiducial markers filled with water and NiCl and technetium-99m which were visible in SPECT and MR images. Scaling and registration of SPECT images on MRI were done based on the markers on a SUN 4/330 computer running NMRi software.

In baboon stimulus(50^{o}C)-control(36^{o}C) SPECT images, increased activity was seen in the contralateral posterior parietal cortex, frontal cortex and the ipsilateral cerebellum. Similar changes were seen in 2 other sessions in anesthetized baboons and macaques. In one human stimulus(46^{o}C)-control(36^{o}C) SPECT session no change was observed. This technique is currently being tested with higher stimulus temperatures in humans and using repeated measures in the baboons to establish statistical significance.

443.3

PSYCHOMETRIC AND SI NEUROMETRIC FUNCTIONS DURING ACTIVE TOUCH OF GRATINGS IN MAN AND MONKEY. R.Sinclair and H.Burton, Department of Anatomy & Neurobiology Washington University School of Medicine, St. Louis, MO 63110. (Supported by NIDCD 00096)

Two *M. mulatta* and 4 humans stroked fingertips over pairs of horizontal gratings, and identified the smoother (smaller groove width). Grating ridge width was 250μm, groove width (GV) varied from 500 to 2900μm. Each grating pair had 1 of 4 standards (500, 1000, 1500, and 2000μm).

Humans discriminated GV differences of ~10%, monkeys ~20%. Neural Weber fractions, for area 3b and 1 cells with graded responses, were computed as firing rate (AFR) change between surfaces divided by AFR to standard. Neural Weber functions paralleled psychophysical ones. Threshold was high for comparisons against the 500μm standard (Stevens' region), and comparably low for other standards (Weber region). In many cells, AFR change for equal GV differences were larger for rough than smooth surfaces, suggesting an exponential relationship between AFR and GV. Large neural Weber fractions resulted for smooth surfaces, explaining skewed psychophysical Weber functions. Discrimination errors were associated with reduction in sensory information in some cells, due to changes in applied force or velocity of stroke.

Thus, cell responses in SI account for ability to discriminate surfaces, and may form the basis for perception of textured surfaces.

443.4

NEURONAL RESPONSES IN SECOND SOMATOSENSORY CORTEX AND VPL OF MONKEYS DURING ACTIVE TOUCH OF TEXTURED SURFACES. H.Burton, R.Sinclair and K.Sathian. Department of Anatomy & Neurobiology and McDonnell Center for Higher Brain Function, Washington University Sch. Med., St. Louis, MO. (Supported by NIDCD 00096)

Recordings were made from 62 SII and 20 VPL neurons in one *M. mulatta* trained to stroke its fingers across and discriminate between two gratings that differed in groove width (0.5-2.9mm). Preliminary results indicate that many SII cells showed little or suppressed activity during contact of their receptive fields (RFs) with gratings. These cells discharged bursts of impulses (1) on initial contact with the surface-bearing block, (2) when contact with the surfaces terminated and (3) when their RFs contacted an elevation between the two gratings on a block. SII cells with pacinian-like responses showed particularly prominent bursts in response to this elevation. Some SII cells displayed response functions that were positively or negatively graded with respect to groove width, and some had response functions that increased with the force of contact during stroking.

Responses obtained from VPL resembled some of those previously described in SI and, like peripheral receptors, showed combined effects of force, velocity and groove width. Relatively high firing rates were observed in some cells in VPL.

443.5

EFFECTS OF A DORSAL SPINAL LESION ON TEMPORAL DISCRIMINATIONS AND ON PHYSIOLOGICAL RESPONSES OF PRIMATE S-I CORTEX. C.J. Vierck[1], B.W. Whitsel[2], J.C. Makous[1], and R.M. Friedman[1]. Dept. of Neuroscience, Univ. of Florida Col. of Medicine, Gainesville, FL 32610[1] and Dept. of Physiology, Univ. of North Carolina School of Medicine, Chapel Hill, NC 27514[2].

Interruption of the ipsilateral dorsal column (DC) in primates spares a variety of spatial capacities (e.g., spatial localization on skin regions supplied by segments caudal to the lesion). However, this lesion produces a substantial deficit on a discrimination of different durations of tactile stimulation (i.e., 3 pulses at 10 Hz vs. longer trains at the same rate). In an effort to understand the neural mechanisms for this reduction of temporal resolution, single unit and evoked potential recordings from the primary somatosensory cortex (S-I) have been conducted in primates. Multiple unit and evoked potential recordings in the DC-deafferented region of S-I reveal activity that waxes and wanes. That is, stimulation does not reliably drive the cortical cells - especially at high rates of stimulation.

Supported by NS 07261 and DE07509

443.6

RESPONSIVENESS OF PRIMARY SOMATOSENSORY CORTICAL NEURONS TO VIBRATORY STIMULI DURING MOVEMENT VS. NO-MOVEMENT TASKS. R.J. Nelson and V.D. Douglas*, Dept. of Anatomy and Neurobiology, College of Medicine, University of Tennessee, Memphis, 875 Monroe Avenue, Memphis, TN 38163.

The responses of some monkey primary somatosensory cortical (SI) neurons to peripheral stimuli may be enhanced when these stimuli trigger movements as compared to when stimuli signal that wrist position should be maintained. It is unclear whether the responsiveness of other SI neurons may be suppressed in movement tasks and whether SI neuronal response enhancement and suppression differ depending upon a neuron's cortical location and receptive field (RF) type. Three adult rhesus monkeys (*Macaca mulatta*) were trained to make wrist flexion and extension movements or maintain their wrist position following delivery of a vibratory stimulus to the palm. Care was provided in accordance with the *NIH Guide for Care and Use of Laboratory Animals, revised 1985*. The activity patterns were analyzed for 6 area 3a neurons, 13 area 3b neurons and 22 area 1 neurons. These neurons were selected because each had short latency (<40ms) vibratory related responses in both tasks, and each had a peripheral RF related to the hand or wrist. Neurons with cutaneous and deep RFs were treated separately. The magnitude of the vibratory stimulus-related activity was measured during movement trials. The vibratory response during no-movement trials was then measured over the same period. Area 3a, 3b, and 1 neurons with deep RFs exhibited a statistically significant ($p<.05$; one factor ANOVA) enhancement in their stimulus-related activity during the movement task as compared with their vibratory response during the no-movement task. Area 3b neurons with cutaneous RFs were also enhanced ($p<.001$) during the movement task. Area 1 neurons with cutaneous RFs showed a significant suppression ($p<.001$) of their vibratory responsiveness during the movement task.

These results suggest that an animal's preparedness to move in response to and/or selective attention toward peripheral stimuli may modulate the sensory responsiveness of certain SI neurons. As well, changes in sensory responsiveness in SI neurons appear to differ depending upon the neuron's cortical location and RF type.

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443.7

A COMBINED MULTIELECTRODE-MICRODIALYSIS PROBE FOR USE IN THE MONKEY CORTEX. A.T. Kulics* and B.A. Donzanti, Northeastern Ohio Universities College of Medicine, Rootstown, OH 44272.

We report here the development of a multielectrode/microdialysis probe which allows repeated penetrations over a long term into various cortical areas in awake, behaving monkeys. The microdialysis technique is used in conjunction with current multielectrode recording techniques (Cauller and Kulics, Exp. Brain Res., 1988). The microdialysis unit consists of an outer hollow-fiber semipermeable membrane and an inner flexible fused silica glass capillary tube. It is housed within 25 ga hypodermic tubing which also contains 3 recording tips spaced 400 μ apart for monitoring electrophysiologic activity. In one experiment, 20 min dialysate samples were collected before and after controlled cutaneous stimulation, and analyzed for amino acids using HPLC-ECD. In another experiment, bicuculline was infused directly through the probe which resulted in reversible disinhibition of the cortical network response to touch stimulation. These data demonstrate the utility of this probe design for the study of neurochemical and electrophysiological changes during localized drug delivery in the cortex of the awake monkey.

443.8

OSCILLATORY ACTIVITY OF SINGLE UNITS IN A SOMATOSENSORY CORTEX OF AN AWAKE MONKEY AND THEIR POSSIBLE ROLE IN TEXTURE ANALYSIS.

E. Vaadia. and E. Ahissar Dep. of physiology, The Hebrew University - Hadassah Medical school, Jerusalem 91010, Israel.

Neuronal activity was extracellularly recorded in the cortex of an awake monkey. Single units displaying oscillatory firing patterns were found in the upper bank of the lateral sulcus in a multi-modal area at the posterior border of SII. The majority of the neurons in the sampled area responded to tactile stimuli. The spectral energies of the oscillating activity were distributed in a tri-modal fashion: 0-15 Hz, 15-50 Hz, 80-250 Hz. The most common frequencies were around 30 Hz. The oscillatory activity was not affected by anesthesia, but was suppressed, in most cases, by tactile stimulation or self-initiated movements. Crosscorrelation analysis of all the neuron pairs (N=364) failed to detect signs of synchronization of the oscillatory activity of different neurons. Analysis of intervals in the spike trains of single neurons suggests that 65% of them were "intrinsic oscillators" while the rest were probably driven by external oscillatory source.

There are reasons to believe that the oscillatory neurons play a role in somatosensory processing: they are located in a somatosensory area; their firing rates and oscillatory components can be affected by tactile stimuli; and the tri-modal distribution of oscillating frequencies resembles the distribution of the three mechanoreceptor types (SA,RA,PC) in the finger tip.

The newly identified oscillators can play a key role in texture analysis if included in a Phase-Locked Loop (PLL) circuit, which measures an input frequency by comparing it to the frequency of a local oscillator. Such a model is presented in a companion abstract.

443.9

TACTILE TEXTURE DECODER MODEL BASED ON CORTICAL OSCILLATORS.

E. Ahissar and E. Vaadia. Dep. of physiology, The Hebrew University - Hadassah Medical school, Jerusalem 91010, Israel.

The texture of a surface is determined by the density (spatial frequency) and the depth (amplitude) of its grooves and ridges. We propose a model that describes decoding of frequency modulation (FM) components of the texture. The heart of the model is a Phase-Locked Loop (PLL) circuit. This circuit, composed of a phase detector (PD) and a rate controlled oscillator (RCO), decodes FM signals and the provided that the input average ("carrier") frequency is equal to the intrinsic frequency of its RCO. Many such PLLs, each tuned to a specific frequency range, operate in parallel within a single "automatic velocity control" (AVC) loop. The role of the AVC loop is to maintain the input temporal carrier frequency in a selected frequency range by controlling the velocity of arm/finger movements. Thus, the model suggests that during surface exploration, the scanning velocity is first voluntarily set to generate temporal frequencies corresponding to the frequency range of a selected receptor submodality (SA, RA, or PC), and then the AVC controls the fine tuning of the finger velocity.

A possible neural implementation of a single PLL is a corticothalamic loop, with three sets of neurons; A set in the thalamic ventrobasal nuclei serves as the PD and drives a set of inhibitory interneurons in the cortex, which in turn drives a set of cortical RCO neurons. The RCO neurons feedback to the PD thalamic neurons. The capability of the thalamic neurons to detect phase differences is achieved by implementing an asymmetrical connection scheme in which the cortical input is mediated by slow excitatory synapses, while the lemniscal (peripheral) input is mediated by conventional fast synapses. The surface frequency modulations are internally represented by the PD neurons population code. A computer simulation supports the feasibility of such implementation.

443.10

HETEROSYNAPTIC AND BEHAVIORAL MODULATION OF FUNCTIONAL CONNECTIONS BETWEEN NEURONS SIMULTANEOUSLY RECORDED IN CORTEX AND THALAMUS J.K. Chapin and H.-C. Shin, Dept. Physiol/Biophys, Hahnemann Univ Sch. of Med., Phila, PA 19102 and Dept. of Anesthesia Res. Lab., Harvard Univ, Boston, MA. 02115.

In previous studies we have shown that sensory transmission to single neurons in the somatosensory thalamus and cortex in the awake rat is dynamically modulated during movement and other behaviors. To define the mechanisms for this modulation we have used spike-triggered cross-correlation techniques to measure dynamic changes in functional connections between neurons simultaneously recorded during these behaviors. Multiple single neuron recordings were obtained through arrays of 9-22 25μ microwire electrodes implanted in the forepaw areas of the SI cortex and VPL thalamus in rats. Serial excitatory connections between neurons were indicated by narrow, short latency post- spike response peaks in the spike-triggered histograms. Even with 20,000 trigger spikes, such peaks were found in only 10-20% of pairs of recorded neurons. Cortico-cortical (C-C) and cortico-thalamic (C-T) connections were found more commonly than thalamo-cortical (T-C) or thalamo-thalamic (T-T). Briefly, the strength of these connections were found to be dynamically modulated during: 1) the refractory period of the post-synaptic neuron, 2) the convergence of two excitatory inputs on the same neuron, 3) stimulation of the peripheral receptive fields of these neurons, and 4) active locomotor limb movements. These modulations were not well correlated with changes in the background discharge of the post-synaptic neurons. These results suggest that neuronal responses to synaptic inputs during behavior are highly non-linear and state dependent, and may involve dendritic processing. *Supported by PHS grants NS26722, AA06965, and AA00089, and AFOSR-90-0266 to JKC.*

443.11

EMERGENT PROPERTIES REVEALED IN MULTI-LAYER NEURONAL NETWORK MODELS: FEEDFORWARD VS. FEEDBACK INHIBITION. J.P. Utz, M.A. Nicolelis and J.K. Chapin. Hahnemann Univ., Phila., PA 19102.

As an initial step in an ongoing effort to produce a computer model of the mammalian somatosensory system, we have investigated the behavior of simple neural circuits which may form building blocks for this system. A neuronal network simulator was developed allowing relatively large numbers of realistic neurons to be connected in multiple layers. These neurons incorporate a membrane potential (V_m) which is calculated from membrane conductances and equilibrium potentials for three ions: Na^+ (for excitation), Cl^- (for inhibition), and K^+ (for afterhyperpolarization). The state variables (eg. for V_m, spikes and synaptic events) are re- calculated, stored, and displayed every msec of network time. During simulation the V_m and spikes of all neurons in both layers are continuously displayed in color mapped arrays. Both models used here incorporated two layers, each containing a 40x40 array of neurons. These were driven by a 40x40 array of randomly timed afferents. Each connection fanned-out in a 5x5 array of synapses. In the feedforward inhibition model, both of the neuron layers received the same excitatory afferents, but layer 2 also received inhibitory inputs from layer 1. During simulation this model produced a relatively flat and random pattern of polarization states in both layers. In the feedback inhibition model, which was inspired by the known connections between thalamus and cortex, layer 1 alone received the afferents and relayed excitation to layer 2, which then fed inhibition back to layer 1. In simulation this model produced distinct globular patches of activity in layer 2, but not in layer 1. These results suggest that feedback inhibitory corticothalamic effects (mediated through the thalamic reticular nucleus) could be partly responsible for the columnar patterns of activity which are well known in cortex. *Supported by grants NS26722, AA06965, AA00089, and AFOSR-90-0266 to JKC and a Dean's Summer Research Award to JPU.*

443.12

CHAOTIC DIMENSIONALITY OF CORTICAL NEURONAL DISCHARGE PATTERNS IS ALTERED BY ANESTHETIC STATE. T.M. Fisher, M.A.L. Nicolelis, and J.K. Chapin. Dept. of Physiol/Biophys, Hahnemann Univ, Phila, PA 19102.

"Chaos" is an ubiquitous feature of non-linear systems producing such natural phenomena as fluid turbulence, population cycles, and variations in the heart rate. The "quasi-periodic" behavior of such systems are often found to converge on a "strange attractor". The fact that the brain contains widespread non-linear inhibitory feedback circuits suggests that it is a likely substrate for chaos. Little is known, however, about how chaos might be expressed in the discharge patterns of individual neurons in the brain, and how this may affect the brain's functionality. This problem was addressed here by analyzing temporal spiking patterns of single units recorded in the cortex of rats during wakefulness and after pentobarbital anesthesia. To assess the possible existence of chaos in these spike trains, temporal patterns of inter-spike intervals (ISIs) were visualized in 2-D or 3-D sequential interval raster (SIR) plots. SIRs of neurons in awake animals tended to show relatively featureless clouds of raster points. After anesthesia SIRs from the same neurons exhibited attractor-like structures. To assess the dimensionality of these SIRs, a standard technique was used in which lines were drawn on a log-log scale plotting the Euclidean distances between points (X-axis) against their correlation integral (C(d); on Y-axis). (C(d) = total number of distances below each value on the Y-axis). A line was calculated for each of a sequence of iterated "embedding dimensions", ie. the number (N) of sequential intervals used to create each point in an N-dimensional space. The dimensionality of the spiking pattern was considered to be equal to the slope of these lines at the point that they stopped increasing along with N. In these analyses, spiking activity from awake animals tended to yield dimensionalities of about 4.5 or greater, some being almost indistinguishable from random noise. By contrast, discharge of the same neurons after anesthesia yielded fractal dimensionality as low as 2-3. These results suggest that the anesthetic state may involve a marked reduction of the number of factors which normally influence cortical neuronal activity. *Supported by NS26722, AA06965, KO2-AA00089, and AFOSR-90-0266.*

443.13

PROPERTIES OF A DISTRIBUTED SIMPLE LINEAR NETWORK THAT LOCATES POINT STIMULI: EFFECTS OF RECEPTIVE FIELD SIZE, SHAPE AND DENSITY. B.J. Serog*, J.W. Holsapple and A.V. Apkarian. Dept. of Neurosurg., SUNY Hlth. Sci. Center, Syracuse, NY 13210.

Determination of stimulus location is a feature common to many sensory processes. It is assumed that the spatial acuity of such processing is determined by the size and density of receptors and that high spatial acuity requires small receptive fields (RFs). For example, nociresponsive neurons in the medial thalamus have large RFs and are assumed to play no role in localization. In contrast, nociresponsive neurons in the lateral thalamus have small RFs and are implicated in localization.

A computer simulation of a linear distributed network that locates point stimuli was studied. The network was composed of: 1) an afferent input layer of "units" of density rho and radius lambda placed on a test surface of unit width and 2) two output "units" whose activity coded position of stimuli in cartesian coordinates. Connection strengths for the network were calculated using an algorithm which optimized point localization. We found that the error in the assignment of coordinates of point stimuli for trained networks varied inversely with the degree of overlap of RFs ($rho x lambda^2$). This lead to the conclusion that large RFs can be associated with high spatial acuity. In addition we found that the profile of RFs had little influence on spatial acuity.

These results indicate that high spatial acuity can be associated with large, overlapping RFs (in a distributed network). The roles currently assigned to medial and lateral thalamic nociresponsive neurons in localization is based on the assumption that the process (network) is non-distributed (labeled line model). The role, however, of nociresponsive neurons, with large and small RFs, in localization depends on the organization of the underlying network (degree of convergence of afferent input) which is unknown for these cells.

443.14

SOMATOSENSORY ORIENTATION-LOCALIZATION AND POSTURAL REFLEX DEFICITS AFTER UNILATERAL SI AND/OR SII CORTICAL DAMAGE IN RATS. R. B. Glassman. Dept. of Psychology, Lake Forest College, Lake Forest, IL 60045.

Ten albino rats were trained, while blindfolded, to turn toward touched points on the body for pieces of sugared dry cereal. As with cats (Glassman, Physiol. Behav., 1977; Physiol. Psychol., 1983, 1985), orientation was more rapid and accurate to anterior points. Unoperated rats were as responsive as cats to tactile stimuli, but much poorer to auditory and visual stimuli. Ablations were carried out by aspiration, with rats under pentobarbital general anesthesia. In most cases SI (n=4) or SII (n=4) was first located with the help of gross evoked potentials to contralateral stimulation of the body. After all behavioral observations, in some cases, evoked potentials were recorded to verify the integrity of remaining somatosensory cortex. Like cats, rats sometimes turned toward the wrong side during early postoperative tests. Damage to SI versus SII in cats had caused doubly dissociated deficits, respectively in movement versus passive touch. Rats showed no such difference between SI and SII damage - either ablation caused deficits in cutaneous orientation as well as in placing and hopping, with severity related to lesion size. The largest, least recoverable deficits (2 months) followed combined damage to SI and SII (n=2).

MOTOR SYSTEMS AND SENSORIMOTOR INTEGRATION: OCULOMOTOR SYSTEM III

444.1

GAZE SHIFTS EVOKED BY SUPERIOR COLLICULUS STIMULATION IN THE ALERT CAT. M. Crommelinck, M. Paré and D. Guitton. Montréal Neurol. Inst. and McGill Univ., Montréal H3A 2B4.

The superior colliculus (SC) is involved in the generation of saccadic gaze shifts. A motor map is found in the SC deeper layers. It was commonly assumed that the site of activity on the map determines alone the saccade vector. Recent data now challenge this notion. Stimulation studies have shown that saccade amplitude varies with current strength. Furthermore, recording and lesion studies have provided evidence that the SC may also encode saccade speed. In the alert cat whose head is fixed or unrestrained, we have studied how varying stimulation parameters affects the properties of evoked gaze shifts. Stimulation was delivered at sites where tectoreticular neurons (TRNs) were recorded. Amplitude and velocity of evoked saccades increased to a plateau value with frequency, current strength and train duration. The amplitude plateau value of the evoked gaze shift equalled the amplitude "prefered" by local TRNs. Noteworthy, the effects on saccade amplitude and velocity were not exclusively interdependent and predictable from the main sequence relation. These effects were observed on either eye saccades evoked from the anterior portion of the SC or eye and head saccades elicited from the caudal part. These results suggest that the SC provides the saccadic command using both the site of activity on the motor map and the amount of activity of the recruited cell population.

444.2

EYE MOVEMENTS IN ESSENTIAL BLEPHAROSPASM. J. L. Demer, J. B. Holds*, and L. A. Hovis*. Jules Stein Eye Institute and Department of Neurology, University of California at Los Angeles, 90024; Department of Ophthalmology, University of Texas Medical Branch at Galveston, 77550.

Frequent complaints of disequilibrium and the finding of abnormal brainstem auditory evoked potentials in patients with essential blepharospasm suggest an abnormality in the lower brainstem. Since an extensive ponto-mesencephalic lesion would also involve ocular motor control areas, we sought abnormalities of eye movements in patients with essential blepharospasm.

We studied 8 patients (mean age 58 yrs, range 44 - 71) who had undergone prior surgery or botulinum injection for blepharospasm; two also had lower face involvement (Meige syndrome). There were two groups of neurologically normal controls: young (mean age 38 yrs, range 30-55) and elderly (mean age 65 yrs, range 50-85). Horizontal eye movements were measured using digitally-sampled, direct current electro-oculography.

All patients had normal saccadic amplitude-velocity relationships for randomly-presented target steps. Vestibulo-ocular reflex gains for multiple frequencies (0.0125-0.4 Hz) of passive, sinusoidal, whole-body rotation in darkness were not significantly different from young controls, and there was normal visual enhancement and fixation suppression. Mean sinusoidal smooth pursuit (0.2 & 0.4 Hz) and full-field optokinetic (0.05 Hz) gains of patients were 20-35% less than for young controls ($p<0.05$). For patients, mean optokinetic gain for 30°/s constant velocity stimulation was ~40% less than in young controls ($p<0.05$), but was not significantly different from elderly controls.

Eye movements in patients with essential blepharospasm do not differ significantly from normal subjects of similar age. This finding is evidence against a diffuse ponto-mesencephalic lesion in essential blepharospasm.

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444.3

CEREBELLAR UVULA CORRELATES OF MOVING VISUAL STIMULI S.J. Heinen* and E.L. Keller. Smith-Kettlewell Eye Res. Inst., San Francisco CA, and Dept. of Elect. Eng., Univ. of Cal., Berkeley, CA.

The uvula (lobule IX of the cerebellar vermis) receives indirect input from the superior temporal sulcus and pretectum via the dorsolateral pontine nucleus, areas all involved in visual motion processing. We were interested in how the uvula might further process these motion signals. We used tungsten microelectrodes to monitor single-unit responses of 108 cells in the uvula of behaving monkeys (n=2) to a variety of moving visual stimuli: sweep of large field textured backgrounds during fixation suppression of optokinetic following eye movements, smooth pursuit eye movement of small moving spots, and buildup of optokinetic nystagmus (OKN) during full field optokinetic drum motion. Drum rotation was the most effective stimulus for activating uvular cells. Most cells (n=57) either increased or decreased firing during one direction of drum rotation, and most of these had a response that was either smaller or opposite in sign for the other direction. The time constant of this change was on the order of 3-5 secs, much faster than long-term buildup of velocity storage during OKN, and this activity persisted in these cells after drum light offset, often outlasting optokinetic after-nystagmus (OKAN). Cells rarely responded to brief large field motion, however, two classes of smooth pursuit cells were found. One class responded briefly when retinal slip was high (60-80 msec after pursuit initiation), and the other class was modulated gradually during the pursuit trial. These results suggest that the uvula is involved indirectly in velocity storage, possibly performing a modulatory function on the velocity storage integrator. (Supported by EY06860)

444.4

PREDICTIVE SACCADES IN HUNTINGTON'S DISEASE (HD) J.R. Tian, D.S. Zee, S.E. Folstein, A.G. Lasker*. Johns Hopkins University, Baltimore, MD 21205

Eye movements were recorded from 21 mildly-affected patients with HD and 20 age-matched controls. All patients made excessive errors on the antisaccade ("look opposite to the target") paradigm. More than 30% of saccades were made incorrectly to the visual target. Patients also showed a defect in generating anticipatory saccades, to a target jumping in a predictable fashion (0.5 hz, 20 deg). Mean saccade latency was 135ms in HD and -78ms in controls. Fourteen of 21 patients had values > 2 standard deviations from the mean of normals. Mean percentage of anticipatory saccades (latency < 100ms) was 28% in HD and 83% in controls. Three patients made no anticipatory saccades at all. Mean amplitude of anticipatory saccades was 14.2 deg in HD and 17.6 deg in controls. Eleven of 18 patients had values > 2 standard deviations from the mean of normals. We conclude that abnormal predictive saccadic tracking is common in early HD and that the basal ganglia probably play a role in generating predictive saccades.

444.5

IMMUNOHISTOCHEMICAL LOCALIZATION OF PARVALBUMIN IN CAT EXTRAOCULAR MOTOR NUCLEI. Robert F. Spencer. Dept. Anatomy, Medical College of Virginia, Richmond, VA 23298.

Previous studies have demonstrated differences in the inhibitory neurotransmitters utilized by functionally equivalent premotor neurones that project to the cat oculomotor and trochlear (GABA) versus abducens (glycine) nuclei. In the sensory visual system (*e.g.*, dorsal LGN, visual cortex) and other areas of the nervous system (*e.g.*, hippocampus), the calcium binding protein parvalbumin (PA) is highly co-localized in certain classes of GABAergic neurones. The immunohistochemical localization of PA in the extraocular motor nuclei was undertaken to asses the role, if any, of PA in GABAergic and glycinergic mechanisms related to the discharge properties of motoneurones during eye movement. In the oculomotor, trochlear, and abducens nuclei, PA-immunoreactive staining was associated with most, but not all, of the cholinergic motoneurones. PA-immunoreactive synaptic endings contained predominantly spheroidal synaptic vesicles and established asymmetric synaptic contacts with both immunoreactive and non-immunoreactive somata and dendrites. The ultrastructural features of PA-immunoreactive synaptic endings are consistent with presumed excitatory synaptic inputs, at least some of which utilize glutamate as a neurotransmitter. Correlated at least in part with the synaptic localization of PA in the extraocular motor nuclei, PA-immunoreactive neurones were located in many of the brainstem preoculomotor structures that project directly or indirectly to the motoneurones. Extrapolating from studies in other regions of the nervous system, it appears that PA is associated with neurones in the oculomotor system that discharge at high frequency. Within the motoneurones, one function of PA may be to buffer the influx of Ca^{2+} that accompanies membrane depolarization following the activation of NMDA receptors by glutaminergic inputs.

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444.6

POOR "SPATIAL" SIGNAL IN HUMAN SACCADIC SYSTEM. R. S. Gellman and W. A. Fletcher* Dept. Clinical Neurosciences, Univ. of Calgary, Calgary, AB, Canada T2N 4N1

To make an accurate saccade to the *spatial* location of an object requires both visual information and a signal encoding eye position at the time that the image appears. To determine if the required eye position signal is available, the experimenter typically evokes an intervening eye movement shortly after presentation of a saccadic target but before a saccade is directed to that target. McKenzie and Lisberger (1986) argued that, in monkeys, the signal is available only if the intervening eye movement is saccadic. When pursuit movements intervened between target presentation and a saccade to that target, the saccades were specified by the visual signal; the intervening pursuit movement was ignored. We report that this dichotomy is absent in human subjects.

Subjects (N=8) tracked a target moving horizontally (15-30°/s) that disappeared after 800-1200 ms. A spot was then flashed for 20 ms 4-12° above or below the meridian, at horizontal offsets of 0-14° from the direction of gaze. Subjects were instructed to look to where the spot had flashed. When subjects looked toward this spot (after 200-300 ms) their saccades compensated for 39% (range 17-55%) of intervening pursuit movement. In a double-step paradigm, where the second step occurred shortly before a saccade to the first target location, and the screen was then blanked, performance improved, but remained poor (compensation 58%; range 43-74%). For intervening saccades or pursuit, compensation varied from 0-100% from trial to trial. Saccades to flashed targets, where no movement intervened, were accurate (gain >0.9 for most subjects).

These results suggest that the human saccadic system use eye position information regardless of whether it is generated by pursuit or saccades. This information is transmitted with low gain and high noise.

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444.7

VELOCITY STEP TRAINING OF THE GOLDFISH VESTIBULO-OCULAR REFLEX SUGGESTS MULTIPLE SITES ARE INVOLVED IN GAIN MODIFICATION. A.M. Pastor*, M. Weiser, S. McElligott and R. Baker, Dept. of Physiol. and Biophys., NYU Med. Ctr., New York, NY 10016, and Dept. of Pharmacol, Temple Univ. School of Med., Phil. PA 19140

Goldfishes were trained to either increase or decrease VOR gain (eye/head velocity) with visual-vestibular stimuli presented in the form of velocity steps. Operant paradigms in which the visual step preceded the vestibular turn-around by 50-150 ms were more effective in producing robust changes in the inital 100 ms of the VOR than either simultaneous visual-vestibular presentation or sinusoidal training. Examination of the eye velocity profile throughout the step response before, during and after either an increase or decrease in vestibular sensitivity revealed an early dynamic and later sustained phase of adaptive plasticity separated by a sag at about 150 ms. Acute cerebellectomy following VOR gain decreases induced with velocity step training did not alter the early dynamic response, but abolished the sag while the remaining constant velocity component was re-scaled close to, but less than, control VOR gain. Within a few weeks the dynamic response returned to normal levels; however, it's latency and amplitude was little, if at all, modifiable up to one year later. By contrast, vestibular sensitivity during the later constant velocity presentation, like that observed with sinusoidal stimuli, could be adaptively modified in some goldfishes. A similar acute response profile was observed with VOR gain increases, except that the latency for the peak of the dynamic response shifted and subsequent VOR gain was usually higher than control measurements. These data argue for more than one site associated with adaptive gain control. If separate channels for information processing are envisioned then the site of plasticity in the vestibular nucleus itself may only be modified when the cerebellar circuitry is intact.

444.8

GENERALIZING THE POST-SACCADIC SLIDE TO SLOW EYE MOVEMENTS IN THE RABBIT. J.S. Stahl and J.I. Simpson. Dept. Physiol. & Biophysics, NYU Med. Ctr., New York, N.Y. 10016.

At the end of a saccade, the firing rate of primate motoneurons continues to decay even after the eye has reached its final position. This exponential decay, termed the post-saccadic slide, has a time constant Ts of approximately 90 ms (Goldstein, 1982). The decay has been hypothesized to compensate for a mechanical lead element in the oculomotor plant; as such the slide is not specific to saccades. It should be present following step changes in eye position produced by other means. We examined neurons of the vestibular ocular system in rabbits for slides following eye movements evoked by changes in head position. Neurons were recorded in awake rabbits and identified as abducens motoneurons, internuclear neurons, or vestibular nucleus premotor neurons by electrical stimulation of the lateral rectus muscle, MLF, or oculomotor complex, respectively. Ramp-step changes in eye position were produced by oscillating the animal about the vertical axis with a trapezoidal position profile (0.5 sec ramp @ 4°/sec, 4 sec stationary, 0.5 sec @ -4°/sec, 4 sec stationary) in the light. Firing rate decayed exponentially during the stationary periods; a time constant Ts was estimated by curve fitting. The distribution of Ts was strongly positively skewed. Median Ts was 1.1 sec (n=17) for the pooled abducens units and 1.7 sec (n=29) for the vestibular nucleus units. These values are 10-20 times larger than those seen in the monkey. In modeling the oculomotor plant of the monkey, a Ts term improves the prediction of firing rates for sinusoidal eye movements above approximately 0.5 Hz (Fuchs et al., 1988). The long slide shown in the present study indicates that the influence of Ts extends to still lower frequencies in the rabbit.

444.9

EFFECTS OF A TEMPERATURE DECREASE ON ADAPTIVE VESTIBULO-OCULAR REFLEX MODIFICATIONS IN THE GOLDFISH J.G. McElligott[1] and R. Baker[2] [1]Dept. of Pharmacology, Temple University School of Medicine, Phila., PA 19140, and [2]Dept. of Physiology and Biophysics, N.Y.U. Medical Center, New York, NY 10016

Previous work in our lab showed that short term adaptive gain changes of the vestibulo-ocular reflex (VOR) in the goldfish were reversibly inactivated by a decrease in temperature of 6 to 12 deg C. Unmodified VOR gain measured in the light and dark was unaffected by a similar temperature change. The work presented here investigates the effect of an acute temperature decrease on acquisition of the VOR change. Goldfish were acclimated to a laboratory aquarium at 21 deg C over a period of several months. After initial calibration measurements of the VOR in light and dark were made, the VOR was modified over a 3 hour period at the acclimation temperature. Training to a gain of 2X was accomplished by presenting visual stimuli 180 degrees out of phase with the vestibular platform rotating about the vertical axis (1/8 Hz ± 20 deg). A second group of fish were trained following the same procedure but VOR modification was carried out at a temperature of 12.5 deg C. This decrease in temperature had no effect on the initial calibration measurements of VOR gain and phase measured in the light (gain = 1) and the dark (gain = 0.9) . The subsequent rate and level of VOR gain change measured in the light and the dark over the 3 hour training period was the same for fishes modified at both temperatures. VOR gain of 2X measured in the light was achieved within the first hour. Gain measured in the dark increased from 0.9X to 1.6X during this period for both groups. No phase changes were detected. This work coupled with our previous study demonstrates that modification of the VOR by the goldfish is achieved with equal facility over a large temperature range. However, a modified VOR gain change acquired at a higher temperature can be reversibly inactivated by a reduction in temperature.

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444.10

TWO-DIMENSIONAL VERSUS THREE-DIMENSIONAL RAPID EYE MOVEMENT GENERATION IN THE SUPERIOR COLLICULUS AND THE ROSTRAL INTERSTITIAL NUCLEUS OF THE MLF IN THE MONKEY. J van Opstal*, V Henn, BJM Hess*, D Straumann, K Hepp*. Neurology Dept, University, CH-8092 Zürich, Switzerland.

Monkeys were chronically prepared with a 3-dimensional search coil, the superior colliculus was localized by single neuron recordings and microstimulation. Nystagmus was tested in 3 dimensions by rotating animals about different axes in the light and in darkness. The superior colliculus was temporarily inactivated by the local microinjection of Muscimol. A unilateral inactivation leads to a deficit of the rate of saccades, their velocity and amplitude in a contralateral horizontal direction together with an ipsilateral deviation of the eyes. A bilateral inactivation leads to a deficit in both horizontal and vertical directions, but does not seem to affect fast phases of torsional nystagmus. This shows that rapid eye movement programming in the superior colliculus involves the horizontal and vertical dimension, but not torsion. It is in striking contrast to deficits observed with lesions of the riMLF, where already a unilateral inactivation leads to clear deficits in torsion.

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444.11

ON THE CENTRAL IMPLEMENTATION OF SACCADES IN LISTING'S PLANE. K Hepp*, J van Opstal*, BJM Hess*, D Straumann, V Henn. Physics Dept, ETH, and Neurology Dept, University Hospital, CH-8091 Zürich, Switzerland.

We have investigated whether the central representation of saccadic eye movements in Listing's plane in the spatial (superior colliculus, SC) and temporal (riMLF) saccade generator is in terms of difference vectors or quaternion quotients of final and initial eye position. The quotient model predicts how SC stimulation drives the eye out of Listing's plane, and that movement fields of saccade-related burst neurons in the SC should have torsional components. With neuronal recording and microstimulation at 60 sites in 4 colliculi of 2 monkeys with a 3D search coil we have found a clear violation of the quotient model. This was confirmed by a significantly better torsional modulation of burst neurons in the riMLF in difference coordinates. These findings suggest that Listing's law for visual saccades is implemented downstream of the spatial and temporal saccade generator.

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444.12

EVIDENCE FOR A FIXATION ZONE IN THE ROSTRAL SUPERIOR COLLICULUS OF THE MONKEY. D. P. Munoz, D. M. Waitzman, and R. H. Wurtz. Laboratory of Sensorimotor Research, National Eye Institute, NIH, Bethesda, MD 20892, USA.

The rostral superior colliculus (SC) of the cat may be involved in the control of visual fixation (Munoz and Guitton, 1989, *Rev. Neurol.*, 145: 567-579). To determine whether an analagous zone exists in primates, we recorded activity of cells in the rostral SC of monkeys trained to make saccadic eye movements and fixate targets. A fixation-related zone was identified with the following characteristics. 1) It was located about 1.5mm below the dorsal surface of the SC, 0.25-0.5mm below cells that discharged high frequency bursts for small (<1° amplitude) saccades. 2) A subset of neurons, fixation-related cells, recorded at this locus discharged tonically when the monkey fixated a small visual target for reward. This activity continued when the fixation target was turned off for 300-600ms and the monkey was required to maintain the same eye position. These cells were only sporadically active during spontaneous fixations, for which the animal was not rewarded. 3) Fixation-related cells paused for saccades to peripheral targets; activity resumed at the end of the saccades. The same pause in activity occurred for saccades to the location of a remembered target (i.e., in complete darkness). 4) When we stimulated the fixation zone with brief, high frequency trains (10-30μA, 2-6 pulses at 500Hz) during saccades, the eyes were momentarily decelerated in midflight at latencies of less than 15ms. 5) Low frequency stimulation (10-30μA pulses at 150Hz) delayed the onset of saccades to targets in all directions. Ipsiversive saccades were initiated only after stimulation offset; contraversive saccades could still be made during stimulation. The results of single neuron recordings and stimulation suggest that collicular fixation cells may serve to gate SC and brainstem premotor circuitry involved in executing saccadic eye movements, reminiscent of brainstem omnipause neurons.

444.13

SACCADE-RELATED BURST CELLS OF THE SUPERIOR COLLICULUS ARE MODULATED BY ELECTRICAL STIMULATION OF ITS ROSTRAL POLE. D. M. Waitzman, D. P. Munoz, L. M. Optican and R. H. Wurtz. Laboratory of Sensorimotor Research, National Eye Institute, Bethesda, MD 20892, USA.

Recent experiments have shown a linear correlation between the decline in burst discharge and dynamic motor error in a subpopulation of saccade related burst neurons in the superior colliculus (SC) (Waitzman et al., *Expl. Brain Res.*, 72:649-52, 1988). To further study the relation between the discharge of these cells and eye movements, we interrupted saccades by microstimulating the rostral pole of the primate SC. Short trains of 2-6 pulses (10 to 30 μA, 500Hz) induced perturbations of visually and memory guided saccadic eye movements at latencies of less than 15 ms. The perturbations ranged from a minor deceleration in midflight (2 pulses) to complete saccade arrest (6 pulses) followed, after short latency, by rapid reacceleration of the eyes and completion of the original saccade onto target. Extracellular recordings of saccade related burst neurons during these perturbations demonstrated that cell discharge was interrupted during the period of stimulation (i.e., during eye deceleration), but activity resumed following stimulation offset, just prior to the reacceleration of the eyes. Cell activity declined linearly with the dynamic motor error until the onset of stimulation. Following stimulation offset cell activity *resumed*, and then declined with motor error as the residual movement was completed. This resumption of activity and its decline were associated with an eye movement amplitude (and also, a residual motor error) for which the cell would not normally fire. A similar pattern of interruption and resumption in activity was occasionally noted during visually guided or memory saccades which *spontaneously* (i.e., without electrical stimulation) decelerated in midflight. These results are consistent with the hypothesis that some of the saccade related burst neurons of the SC are within the brainstem feedback loop controlling saccade amplitude and encode dynamic motor error.

444.14

VELOCITY VERSUS POSITION FEEDBACK TO THE SUPERIOR COLLICULUS IN GAZE CONTROL MODELLING.
Ph.Lefevre[1]*, H.L.Galiana[2]. [1]Lab.of Neurophysiology UCL av.Hippocrate 54 1200 Brussels Belgium; [2]Dept.Biomed.Eng., McGill, Montreal H3A 2B4.

The notion of gaze position feedback is now widely used in all models of natural eye-head orienting movements [1]. This idea was first an extension of the Zee et al. model of saccadic eye movements control, where an efferent copy of eye position was fed back and compared to desired eye position in order to control the saccade amplitude. Previous models always consider gaze position feedback as acting downstream to the SC (superior colliculus), so that from the SC point of view, the generation of saccades is an open loop mechanism. In contrast, a recent model [2] assumes that the SC is **inside** the gaze control loop, so that dynamic gaze error is continuously updated within the SC itself.

Here, we intend to present the comparison of gaze position versus gaze velocity feedback to the SC. The consequences of each control mode have been investigated in terms of: (a) Control accuracy (b) The different collicular networks supporting these strategies and their respective connectivity, and (c) Spatio-temporal transformation in the SC, and reverse temporal-spatial mechanisms. The net result of this study is the implementation and simulation of a collicular neural network performing both spatio-temporal and temporal-spatial transformations in the one dimensional case (for horizontal movements); this model, based on velocity feedback, achieves both spatial and temporal integration inside the SC, and is extremely efficient with regard to its connectivity and cell background activities (energy). [*Supported by SPPS Belgium & MRC Canada.*]

[1] Laurutis & Robinson: J.Physiol.373,pp.209-233,1986
[2] Guitton, Munoz & Galiana: J.Neurophysiol. In press 1990.

444.15

SIGNALS RECORDED IN PRIMATE SUPERIOR COLLICULUS WITH SACCADES EVOKED BY FRONTAL EYE FIELD MICROSTIMULATION. M. Schlag-Rey, J. Schlag and P.Dassonville. UCLA, BRI and Dept. Anatomy & Cell Biology, Los Angeles, CA 90024.

Projections from movement and visuomovement cells of the frontal eye field (FEF) to the intermediate layers of the superior colliculus (SC) have been demonstrated by antidromic stimulation (Seagraves and Goldberg, 1987). We sought to determine the nature of the FEF signal reaching SC movement cells in monkey. First, movement fields of cell pairs (one FEF cell, one SC cell) were determined by unit recording during the performance of visual and saccade tasks. Then, the FEF site was stimulated (10-20 pulses of 0.2ms at 400 Hz, 5-30 μA) while recording from the SC cell. Preliminary results indicate that: (1) SC cells were excited by FEF stimulation when their preferred movement vectors were similar (movement field overlap). (2) Peak excitation seemed better related to saccade onset than stimulation onset. (3) However, excitation was still present, although weaker, at subthreshold intensities for evoking saccades. (4) FEF stimulation inhibited SC cells whose movement field did not include the vector of the evoked saccade. (5) The excitatory or inhibitory nature of the SC response depended on the site of stimulation in FEF, but not on the actual saccade vector when the latter was modified by the saccade collision paradigm. (USPHS grants EY02305 & EY05879 and NSF grant RCD87-58034)

444.16

INTRINSIC CIRCUITRY IN THE CAT SUPERIOR COLLICULUS IS HIGHLY DISTRIBUTED. M. Behan and P.P. Appell*, Center for Neuroscience and Dept. Comparative Biosciences, University of Wisconsin, Madison, WI 53706.

There is a complex network of axonal arborizations and boutons labeled following small, localized injections of *Phaseolus vulgaris* leucoagglutinin into the superficial layers of the cat superior colliculus. Labeled boutons are found in all regions of the dorsoventral, mediolateral and rostrocaudal extent of the colliculus, irrespective of whether the injections are placed in the collicular representation of the area centralis or of the peripheral visual field. There is a tight distribution of boutons in the superficial layers surrounding the injection site. However, there are certain differences in distribution of labeled boutons in the deeper layers depending upon where in the visual field representation injections are made. With a central field injection, the concentration of labeled boutons is greatest directly ventral and rostral to the injection site. Following an injection into the superficial layers in a peripheral field representation, there is a more dispersed distribution of labeled boutons in the intermediate and deep layers, with a distinct rostral polarity. These local differences in connectivity may provide the anatomical substrate for population coding of saccadic eye movements in the superior colliculus.

444.17

A DAMPED REPRESENTATION OF EYE POSITION IS USED IN OCULOMOTOR LOCALIZATION. P. Dassonville, J. Schlag, and M. Schlag-Rey, UCLA, BRI and Dept. of Anatomy, Los Angeles, CA 90024.

Since the study by Hallet & Lightstone (1976) using a double-step paradigm of target presentation, it has generally been assumed that the oculomotor system is accurate in targeting a flash occurring immediately before, during or after an intervening saccade. Their findings differ from the many studies which show inaccurate *perceptual* localization of a flash occurring near the time of a saccade (e.g. Matin 1976, Mateeff 1978).

Honda (1989) has recently shown that the oculomotor system is not as accurate at compensating for intervening saccades as previously assumed. Data from our laboratory, using the colliding saccade paradigm, have also indicated oculomotor inaccuracies (Dassonville 1990), demonstrating that the artificial retinal error created by microstimulation of the primate frontal eye field is converted to either a spatial error by adding an eye position signal that is a damped version of the actual saccade, or a motor error by subtracting a damped version of the change in eye position.

In the current study, two human subjects, in complete darkness, were required to make a saccade to the location of a 2 ms flash (S2) occurring near the time of an initial, visually-evoked saccade to a previous 5 ms flash (S1). A monocular magnetic search coil was used to measure saccade accuracy. Under these conditions, the subjects mislocalize S2 in a manner similar to that seen in Honda's and Mateeff's perceptual studies. The internal representation of eye position was found to be a damped version of the initial saccade (similar to that found with the colliding saccade paradigm), with a time constant of 50-120 ms. Flashes presented immediately before the initial saccade were mislocalized in the direction of the saccade, while those presented immediately after were mislocalized in the opposite direction. Similar results were found when the initial saccade was self-initiated with S1 not presented, removing any chance of an allocentric solution to the task. Methodological differences can at least partly explain the dissimilarity of our results (as well as those of perceptual studies) and those of Hallet & Lightstone, whose task could be solved by the use of the allocentric relationship of the stimuli.
(NSF grant RCD87-58034 and USPHS grants EY05879 & EY02305)

CONTROL OF POSTURE AND MOVEMENT: ARM AND HAND

445.1

INERTIAL MASS LOADING AND THE FREQUENCY RESPONSE FUNCTION FOR CYCLIC, SELF-PACED HAND MOTION. R. N. Stiles and D. W. Hahs*, Dept. Physiology and Biophysics, Univ. Tenn., Memphis, Memphis, TN 38163.

While increasing the inertial mass of the human hand reduces the mechanical resonant frequency of the muscle-hand system (i.e., the physiological tremor frequency), the effect of mass on cyclic voluntary hand motion is uncertain. Pulse perturbation results also indicate that the muscle-hand system behaves as a lightly damped, second-order mechanical system, resulting perhaps from muscle-tendon elasticity, hand mass, and joint viscosity. However, the muscle-hand frequency response for cyclic voluntary motion indicates third-order mechanics with break frequencies at about 1 Hz and 8 Hz.. This suggests first-order muscle mechanics (with a 15-20 ms delay for force generation) in series with the lightly damped, muscle-tendon-mass system. We studied the effect of mass loading on the frequency response (relating hand displacement and surface EMG envelope amplitude as a function of frequency) of the muscle-hand system for cyclic, self-paced, extension-flexion wrist motion. The frequency range studied was 1-10 Hz, with peak angular hand displacement at the fundamental (intended) frequency occurring between about 5-10 deg. The maximum load of 500 gm had a small but predictable effect on the amplitude-frequency plot, increasing the negative slope between 5-10 Hz. With 500 gm, the EMG-Displacement phase plot was shifted, with phase lag increasing by about 50 deg at 8-10 Hz. When considered in the time domain, the effect of mass on the kinematics of quick, point-to-point hand movements was small, and appeared to be mainly due to changes in the lightly damped, second-order sub-system.

445.2

MAXIMAL VOLUNTARY CONTRACTION AND FORCE MATCHING IN THE THUMBS USING A MULTIDIRECTIONAL DYNAMOMETER. R.Forget, D. Bourbonnais, B. Lamarre* and L. Carrier*. Institut de readaptation and Ecole de readaptation, Universite de Montreal, C.P. 6128 succ A., Canada H3C 3J7

Two multidirectional dynamometers were used to record the maximal isometric forces (MIF) exerted at the proximal phalanx of each thumb and to measure the forces developed during a simultaneous matching task of 10% MIF with the contralateral thumb. The maximal forces exerted by 12 normal female subjects (23+1.5 years) with their dominant (D) and non-dominant (ND) sides were measured twice (2 sessions within a two weeks interval) in 8 directions covering 360° by steps of 45° in the transverse plane of the longitudinal axis of the thumb.

The MIF of largest magnitudes were obtained in the directions that brought the thumb towards the palm of the hand (flexion and adduction). No differences were found between the two recording sessions for these directions. There was no strength differences between the D and ND sides.

Force matching was also more precise in the more functional directions towards the palm. Errors in matching the forces angle and magnitude were both correlated with the errors in precision. However, errors in angle were different depending on the direction (largest in the abd./flex.). The precision of matching was similar for the D and ND sides. (Funded by the FRSQ)

445.3

MODULATION IN PREHENSILE FORCE WITH POSITIVE AND NEGATIVE LOAD FORCES. L.A. Jones[1] and I.W. Hunter[2], School of Physical and Occupational Therapy[1] and Dept. Biomedical Engineering[2], McGill University, 3654 Drummond St., Montreal, Canada H3G 1Y5.

The forces used to grasp an object have been shown to vary linearly with the weight of the object, and the slope of this relation is inversely proportional to the friction between the skin of the hand and the object being held (Westling & Johansson, Exp Brain Res 1984, 53:277-284).

The objective of the present experiment was to measure the modulation in prehensile force when negative (pull) and positive (push) load forces were imposed on the hand and to examine how these pinch forces changed as a function of the friction between the skin and the manipulandum being grasped.

Subjects held a manipulandum, which was composed of two symmetrically mounted disks, between the tips of their thumb and index finger. The manipulandum was connected to the stage of an electromagnetic linear motor that generated (under computer control) positive and negative forces. The position of the manipulandum was fed back to subjects on a visual display and they were required to maintain this constant while the forces generated by the motor increased in a ramp from 0 to 30 to 0 N, in both the negative (pull) and positive (push) directions. The forces produced by the motor and the fingers, and the position of the manipulandum were recorded on-line by a MicroVAX computer during each 100 s trial. Four different manipulandum surfaces, each with a different coefficient of friction, were used.

The changes in pinch force were tightly coupled to the forces generated by the motor in both the positive and negative directions, although for many subjects this modulation was asymmetric in that much greater pinch forces were generated to counteract negative load forces. As reported previously, pinch forces varied with surface structure, with larger forces being produced when the friction was smaller.

445.4

MODERATELY IMPAIRED HAND SENSIBILITY AFFECTS GRASP FORCE REGULATION. K.J. Cole. Dept. Exercise Science, The University of Iowa, Iowa City, IA 52242.

The role of tactile signals in regulating grasp force apparently is reflected in reports of excessive grasp force in patients with severe sensibility losses, and in the elderly, who typically show moderate sensibility impairments. However, the contribution of sensibility loss to excessive grasp force is not clear and requires experimental control of age, hand sensibility, and hand friction. Moderate sensibility impairment was progressively induced in subjects who wore surgical gloves of increasing thickness. Some subjects underwent anesthetic nerve block of the thumb and index finger as well. Sensibility was measured using vibrotactile and two-point stimuli. With moderate sensibility losses, subjects exhibited one of three behaviors when lifting the test object: 1) excessive grasp forces, as hypothesized; 2) safety margins decreased substantially, apparently reflecting an impaired ability to monitor grasp forces; or 3) safety margins decreased or did not change, but subjects reduced the vertical acceleration of the object and limited the object's inertial load on the hand. Grasp force regulation is affected by even moderate sensibility impairments, but increased safety margins may reflect a consistently applied strategy only after chronic, rather than acute sensibility losses. Increased hand slipperiness may also contribute to developing this strategy of using excessive safety margins.

445.5

DISTANCE AND LOCATION VARIATIONS AND THE CONTROL OF RAPID BIMANUAL MOVEMENT. D.E. Sherwood. Motor Behavior Lab., Department of Kinesiology, University of Colorado, Boulder, CO 80309.

When subjects make rapid bimanual aiming movements over different distances, assimilation effects are shown. The longer-distance limb usually undershoots its target, while the shorter-distance limb overshoots. However, in most of the studies showing assimilation effects, movement distance and end location have been confounded (i.e., the limbs move different distances to different target locations). In order to resolve this issue, 60 male and female students moved a light, aluminum lever in the sagittal plane with each hand to same or different target locations with either the same or different starting point. Assimilation effects were shown as overshooting in the left hand when either location or both distance and location were varied. The assimilation effects were reduced over 50 practice trials with knowledge of results (KR), but were also noted on a no-KR transfer phase of 25 trials. The results suggest that velocity or muscular force may be programmed by the motor system, rather than distance or location per se.

445.6

BIMANUAL COORDINATION IN TYPING. J.F.Soechting and M.Flanders. Dept. Physiology, University of Minnesota, Minneapolis, MN 55455.

Typing is an ideal model system to study many aspects of skilled movement. It involves coordinated movement of the fingers of both hands. It is a serial process with a well-defined goal (the depression of a set of keys in sequence), but its execution can involve parallel processes (the movement associated with one keystroke can begin before the previous key has been struck). The movements can be measured with relative ease.

We have begun to study typing movements by asking subjects to type words in which all but one letter is typed with one hand, thus permitting us to define the prototypical movement associated with a single keystroke of the other hand. We characterize the movement in terms of linear translation and angular rotation of the wrist, the change in length of each of the fingers (measured from metacarpal joint to finger tip) and the angular orientation of the fingers. We find that each letter is characterized by a unique pattern of movement (often involving all of the fingers of one hand). This pattern is independent of the movement of the other hand.

Supported by USPHS Grant NS15018.

445.7

LEFT AND RIGHT HAND DIFFERENCES IN POINTING TO PERTURBED TARGETS. H. Carnahan and M.A. Goodale. Dept. of Psychology, Univ. of Western Ontario, London, Ontario, Canada, N6A 5C2.

The purpose of this study was to determine how rapidly subjects can amend movement trajectories of the left and right hands in response to unexpected target movement. On separate blocks of trials, subjects reached out and pointed with the left or right index finger to a target light that either remained in a central position, or was changed to a new location on the left or the right, upon release of the start button. Movements of the index finger were monitored by a WATSMART system.

There was no evidence for very early trajectory corrections (before peak velocity) for either hand, nor were there any hand differences in terminal error, RT, or time of correction. For the left hand however, MT was longer, peak velocity was lower, and time to peak velocity occurred later, when compared to the right. These findings also interacted with target location. These results are discussed in terms of hemispheric specialization in motor control. (Supported by MRC grant MA-7269 to M.A.G.)

445.8

HAND DIFFERENCES IN PREHENSION: A KINEMATIC APPROACH. B. Sivak and C.L. MacKenzie. Canadian Centre for Habilitation and Research and Department of Kinesiology, University of Waterloo, Waterloo, Ontario, Canada, N2L 3G1.

This study examined prehension differences between the hands. Right-handed subjects were instructed to reach for and grasp an object placed in front of them. Two factors were manipulated 1) hand (left, right) and 2) type of grasp (collective, independent finger grasp). Analyses involved a quantitative examination of the kinematics of movement as related to transport and grasp components. With respect to the transport component, analyses revealed no differences between the limbs. However, there were differences related to the type of grasp used. Peak velocity occurred later for a collective finger grasp than for an independent finger grasp regardless of the hand used. For the grasp component, subjects opened the hand wider with the left hand than the right hand for an independent finger grasp. There were no differences between the hands in the size of the hand opening when a collective grasp was used. The results suggest that hand differences may be related to the processing of visuomotor information subserving independent finger control.

(supported by NSERC Grant # OGP8303)

445.9

THREE-DIMENSIONAL (3D) ISOMETRIC FORCES EXERTED BY HUMAN SUBJECTS. J. T. Massey, J. T. Lurito, and A. P. Georgopoulos. Bard Laboratories of Neurophysiology, Department of Neuroscience, The Johns Hopkins University School of Medicine, Baltimore, MD 21205.

Human subjects exerted X-Y-Z forces on an isometric handle with the unrestrained arm to move a cursor in the direction of a visual stimulus presented in an anaglyphic (3-D) display (Massey et al., J Neurosci Methods 26:123, 1988). Naive subjects were unable to perform the task with precision. Long term studies were undertaken with three subjects. The 3-D directional variability of the forces exerted and and variance about the stimulus direction decreased with practice and reached an asymptote after approximately 4,000 trials; the half-angle at the apex of the 95% confidence cone for mean direction was approximately 10° at this time. Reaction times fluctuated widely during the earlier trials but attained relatively uniform values as skill increased. These findings suggest that a substantial perceptual-motor learning is involved in generating 3D isometric forces based on information derived from stereoscopic displays. (Supported by NSF BNS-8810642 and ONR N00014-88-K-0751.)

445.10

RADIUS OF CURVATURE AND TANGENTIAL VELOCITY COVARY IN CONTINUOUSLY EXERTED THREE-DIMENSIONAL (3D) ISOMETRIC FORCES J.T. Lurito, J.T. Massey, and A.P. Georgopoulos Bard Labs, Dept of Neuroscience, The Johns Hopkins Univ. School of Medicine, Baltimore, MD 21205.

Viviani and Terzuolo (*Neuroscience* 7:431,1982) showed a piecewise linear relation between the tangential velocity and the radius of curvature of continuous 2D arm movements in human subjects. That is, the tangential velocity was lowest when the path was most curved. This suggested a basic principle that relates movement form and dynamics, namely that "the trajectory goes through equal angles in equal times". The authors proposed that this principle arises from "the logic of the central control processes" rather than from the biomechanical properties of the moving arm.

We tested this idea during exertion of 3D isometric forces, in which case biomechanics play a greatly reduced role in performance. Twenty human subjects grasped a 3D isometric manipulandum (*J Neurosci Methods* **26**:123,1988) with an unrestrained, pronated arm and exerted forces continuously to trace or draw from memory ellipses and lemniscates in specified planes with or without a 3D visual force feedback cursor. Under any of these conditions and in all subjects, we observed a significant positive correlation between the radius of curvature and tangential velocity; that is, when the force trajectory was most curved, the force tangential velocity was lowest. Thus, our observations support the notion that central constraints give rise to the relation between movement trajectory and dynamics. (Supported by NSF BNS-8810642 and ONR N00014-88-K-0751.)

445.11

ACTIVITY PATTERNS OF ARM MUSCLES ASSOCIATED WITH RAPID WRIST MOVEMENT IN MAN. F.Aoki Department of Rehabilitation Research, Tokyo Metropolitan Institute of Gerontology, Itabashi-ku, Tokyo 173.

Adjustment of arm posture associated with rapid wrist movements was studied by EMG analysis. Seven healthy adults, sitting and holding their right arm in mid-position at the shoulder and in 90° flexion at the elbow, were instructed to flex(F) or extend(E) the wrist as fast as possible. To examine whether the activity patterns of the upper arm muscles were related to the prime mover or the direction of the movement, the forearm was in two postures, supinate(Sup) and pronate(Pro). The surface EMGs of biceps brachii(Bi), brachialis(Br), triceps brachii(Tr) and the prime mover, flexor carpi or extensor carpi were recorded along with the angular displacement of the wrist. The sequences of the EMG onsets of the upper arm muscles were changed with the combination of the forearm posture and movement:Tr→Bi for Sup-E, Bi→Tr for Sup-F, Br→Tr for Pro-E and Tr→Br for Pro-F in all subjects except one in whom the pattern for Sup-E was Bi→Tr. The durations from the onset of the earliest activity of the upper arm muscles to that of the prime mover were 4.5±9.3(M±S.D.) ms, 1.8±10.0 ms, 0.8±9.0 ms and -4.5±9.8 ms in Sup-E, Sup-F, Pro-E and Pro-F respectively. From these results, I conclude that the activity patterns of the upper arm muscles are related to the direction of the movements, and that the earliest activity of the upper arm muscles acts in the appropriate direction to counterbalance the dynamic perturbation induced by the movement. And they are considered to be controlled by feed-forward mechanism.

445.12

HUMAN EYE, HEAD AND ARM ROTATIONS DURING REACHING AND GRASPING. D Straumann, K Hepp*, MC Hepp-Reymond, T Haslwanter* Neurology Dept, University Hospital, Physics Dept, ETH, and Brain Res Inst, CH-8091 Zürich, Switzerland.

Using the search coil technique, we have measured simultaneously 3-dimensional eye, head and arm rotations in human subjects during various reaching and grasping tasks. Rotation axes of eye, head and arm were all confined to Listing planes with small torsional components. For eye-in-space (= gaze) and head-in-space rotations, torsional standard deviations were in the range of 0.5 - 1.5 deg, for rotations in the humero-scapular joint between 2.0 and 3.5 deg. The normal vectors for the gaze and head planes were directed frontally and almost in parallel, regardless of the task. The orientation of the arm Listing plane depended on the reaching and grasping paradigm due to the distribution of torsion between the upper and lower arm. These data suggest that the neural network organizes all 3-dimensional rotatory systems in planes with fixed alignment, thus allowing linearization of synergies.

Supported by SNF 3.503-0.86 and EMDO.

445.13

THE AXIS OF ROTATION OF THE ARM DURING POINTING. J. Hore, M. Goodale, and T. Vilis, Depts. of Physiology and Psychology, University of Western Ontario, London, Ont. Canada N6A 5C1

The orientation of the arm in three dimensions was measured in normal human subjects while they pointed to successive visual targets (max range 90°). A six foot diameter search coil system was used with a coil taped to the subject's wrist, such that the overall action of all joints was assessed. The rotations of the wrist from a central initial position formed an approximate planar surface in three dimensional rotation space. This surface was similar to Listing's plane for the eye, (Tweed and Vilis, Vision Res 30: 111-127, 1990) but was thicker, i.e., the torsional orientation of the wrist in space was more variable (S.D. ± 5°). Two factors contributing to the variability for a particular pointing direction, were a slow drift in the orientation adopted by subjects and a dependence on the location of the previous target. The planar surface was not the result of muscle mechanics because the subjects could be instructed to adopt a different wrist orientation which resulted in a shifted planar surface.

The axis of arm rotation relative to space was fairly constant when pointing between target pairs. This axis shifted in space depending on the pointing direction of the arm. For example in pointing from left to right a vertical axis was used which tilted up when the arm was raised and tilted down when the arm was lowered, in both cases by approximately half the angle. Thus the rules of rotations for the arm when pointing appear to be similar to those used by the eye. Support by an MRC grant to J. Hore MT-6773.

445.14

A KINEMATIC ANALYSIS OF JOINT MOTION DURING CURVILINEAR POINTING MOVEMENTS. T.R. Kaminski & A.M. Gentile. Teacher College, Columbia Univ., New York, NY 10027

The hypothesis investigated is that coordinated motion at the elbow and shoulder joints during both linear and curvilinear movements is based on a uniform strategy. Curved, point-to-point movements were performed by having the hand pass over an intermediate point between the initial position and the target.

Results from 8 adults revealed that the shape of the shoulder velocity profile remained essentially the same (smooth with one peak); even in instances where the hand velocity profile had multiple peaks. In contrast, elbow motion frequently demonstrated directional reversals and the velocity profile was dependent on the amplitude of displacement before and after passing the intermediate point. Inflections in the hand velocity profile occurred only when there was a large change in the difference in joint displacement (shoulder vs. elbow) relative to the intermediate point.

These results suggest that the joint control strategy is similar for both linear and curvilinear movements. In both cases, the shoulder provides a stable base for the organization of the movement. Furthermore, hand trajectories cannot be divided into multiple movement segments based on inflections in the hand velocity profile. These inflections maybe the result of a mechanical interaction or the need to maintain coordination between the joints.

445.15

EQUILIBRIUM VECTOR SPACES FOR THE CONTROL OF MULTI-MUSCLE SYSTEMS. A.G. Feldman, J.R. Flanagan, and D.J. Ostry. Institute for Information Transmission Problems, Moscow, U.S.S.R. and McGill University, Montreal, Canada.

A further development of the equilibrium point hypothesis (λ model) is presented with the focus on the control of multi-muscle systems during goal-directed movements. According to this hypothesis, control is associated with changes of neurophysiological parameters (λs) which define the equilibrium state of the system. Muscle activations, forces and movement arise as a natural dynamic reaction of the system to the shift in the equilibrium. Changes in λs for a set of muscles can be coordinated directly by central commands or conditioned by the intermuscular interaction mediated by muscle afferents and interneurons. The afferent interaction is also under central control. In the λ model, multi-muscle commands are represented by vectors. Each vector is associated with a linear combination of λ parameters for a set of muscles and its length represents the strength of the corresponding command. In this space, there are basic vectors which represent functionally different types of coordination. For example, one vector command produces activation of muscles without changes in arm position while another may control motions about an individual joint without other joint motions. Any other control signals can be represented as a linear combination (superposition) of the basic control signals. Two versions of the model are presented, one for goal-directed arm movements and the other for mandible movements. Both versions reproduce characteristic kinematic and EMG patterns of natural movements.

445.16

EQUILIBRIUM VECTORS UNDERLYING MOVEMENTS TO VISUALLY DISPLACED TARGETS. J.R. Flanagan, A.G. Feldman and D.J. Ostry. McGill University, Montreal, Quebec and Institute for Information Transmission Problems, Moscow, U.S.S.R.

The form of central commands underlying movements to visually displaced targets was examined within the framework of the equilibrium point (EP) hypothesis (λ model). According to this hypothesis, movement results from shifts in the equilibrium state of the motor system associated with the dynamic interaction of central commands, reflex mechanisms, muscle properties, and loads. Subjects produced pointing movements to targets located in a horizontal plane. The start of each movement was signalled by the illumination of an initial target. After a delay, the initial target was turned off and a second (displaced) target was illuminated. The position of the first target and the onset, amplitude, and direction of the displaced target were varied. The 3-D trajectory of the hand was recorded. Computer simulations based on the λ model were conducted to compare predicted trajectories, generated from theoretical central commands, with experimental trajectories. Previous reports have suggested that when a visual target is displaced, movement direction is continuously adjusted between the initial and final target (Sonderen, J.F. et al., *Exp. Brain Res.*, 78:139, 1989). Moreover, it has been argued that this process may involve the superposition of the trajectories to the two targets (Henis, E. and Flash, T., *Percept.*, 18, 495, 1989). We suggest that, at the planning level, central commands specify the direction and rate of shift (i.e., velocity vector) of the hand's equilibrium position. When the target is displaced, the equilibrium vector is simply shifted towards the new target.

445.17

ROLE OF HEAD INITIAL POSITION IN THE DIRECTIONAL CODING OF REACHING. S. Vanden Abeele*, V. Delreux* & A. Roucoux. Lab. de Neurophysiologie, Louvain Univ. Sch. of Med., 1200 Brussels, Belgium.

It has been postulated that goal directed arm movements require an internal representation space to which both sensory and motor signals are referred. This interface implies the transfer of the position of the target's image into a body-centered system of reference. This operation takes into consideration the position of the eye in the orbit (Delreux & al. Soc. Neurosci. Abstr., 1989). In the present set of experiments, the role of head position on the trunk has been investigated.

Human subjects sat facing a hemispheric screen, and were asked to move a handle in the direction of LED's presented at several positions along the horizontal meridian. The trunk was not allowed to move. There were four initial conditions for eye and head: 1. eye and head aligned on the midline; 2. eye and head aligned at 15°; 3. head on the midline, eye at 15°; 4. eye on the midline, head at 15°. In each of the four conditions, the subjects were either asked to orient their eyes towards the peripheral targets or to keep them in the initial position. The subjects oriented their forearm towards four peripheral targets (25 to 40°), in complete darkness.

In the eye mobile conditions, the analysis of the distribution of final arm position errors suggests that the reference used is the trunk antero-poterior axis. In the eye immobile conditions, data suggest that a retinal frame of reference is used, though initial head position influences final arm position.

In conclusion, it appears that, in our conditions, the final position and not the amplitude of the pointing arm movement is controlled. This control operates within a body-centered frame of reference; but the final adjustments responsible for the final error rely on mechanisms that may differ, according to the available information about target, eye and head position .

445.18

IS FOREARM ORIENTATION PERCEIVED MORE ACCURATELY THAN ELBOW JOINT ANGLE? W.G. Darling, Dept. of Exercise Science, University of Iowa, Iowa City, IA 52242.

A number of studies have shown that the angle of the forearm in extrapersonal space is perceived better in relation to axes external to the upper limb than in relation to the axis of the proximal arm segment. Exactly how such extrinsic angles are perceived has received little study. The perceptual task was simplified in previous studies by restricting either the angle of the arm segments or the plane of motion of the matching limb. In the present study subjects matched a remembered reference forearm or elbow angle (elbow flexion, forearm yaw or forearm elevation) with flexion or extension motion at the elbow only. The angle and plane of the arm segment during matching was different than in the reference position so that subjects could not simultaneously match the elbow and forearm angles. Elevation matching produced low constant and variable errors, supporting the theory that forearm angle is perceived accurately in relation to the gravitational axis. Matching of elbow angles produced larger variable errors and yaw matching produced larger constant errors. Subjects stated that elbow angle matching was "easiest" and that they used mental imaging of the forearm and its angle relative to imagined vertical and horizontal axes passing through the elbow to match the elevation and yaw angles. These results indicate that subjects use processes of mental imaging and rotation to perceive limb segment angles in extrapersonal space.

445.19

WHAT ARE THE UNITS OF MOTOR BEHAVIOR: PSYCHOPHYSICS OF HUMAN ARM TRAJECTORIES. E.P.Loeb. Department of Brain and Cognitive Sciences, Massachusetts Institute of Technology, Cambridge, MA 02139.

It has long been known that rapid sequences of key-presses or spoken words exhibit a strong linear relation between the number of sequence elements and the reaction time to the beginning of execution of the first element. The goal of this study was to determine if there is a strong linear relation between reaction time and kinematic features of rapidly produced drawings. Normal right-handed subjects drew simple shapes (triangle, square, star) or made oscillatory motions (3, 4, 5 lines) according to standard reaction time methods. The number of peaks in the velocity profile of each drawing was used as the kinematic correlate. A linear relation between velocity peaks and reaction time was found for the shapes, but not for the oscillatory motions. This finding suggests that oscillatory motions and line segments are primitive units of motor planning or behavior.

445.20

MEASUREMENT OF THE ERROR DEADZONE AMPLITUDE IN HUMAN VISUO-MOTOR TRACKING. (SPON: Brain Research Assoc.). R.C. Miall*, D.M. Wolpert* & J.F. Stein*. University Lab. Physiology, Parks Road, Oxford OX1 3PT, UK

Human arm movements when tracking visual targets are often intermittent, suggesting that an error deadzone is involved, i.e. a threshold that must be crossed before a corrective movement occurs. To test this, we have introduced an additional artificial error deadzone (AED) and measured the smallest size of AED which leads to impaired tracking performance.

Subjects tracked a pseudorandom waveform with a small hand-held joystick. The target was a stationary square in the centre of a computer screen. The joystick cursor was displaced horizontally from the target by the error between the joystick position and its desired position, as defined by the pseudorandom waveform ('compensated tracking'). In each trial, 1 of 10 AED amplitudes between 0 and 20 pixels was introduced (0 - 1.26°). If the positional error was less than the current AED, then the cursor was displayed exactly on-target; at all other times its position reflected actual joystick position. We then computed the subject's integrated error score for the last 25s of each 30s trial, and plotted the average for each AED amplitude. Tracking performance varied considerably between subjects; smaller variance was seen between repeat trials by individuals. However, performance errors were approximately constant for each subject with small AEDs (≤ 6 pixels or 0.44°), but rose linearly above this threshold.

These results indicate that an error deadzone of about 0.44° measured at the eye contributes to the intermittency of visually guided arm movements.

445.21

FORCE REQUIREMENTS DETERMINE THE PATTERN OF AGONIST MODULATION. D.S. Hoffman, M.R. Stiles* and P.L. Strick. VA Med. Ctr. and Depts. of Neurosurg. & Physiol., SUNY-HSC @ Syracuse, Syracuse, NY 13210.

There has been controversy regarding the factors producing height and/or duration modulation of the agonist burst during step-tracking movements (Gottlieb et al., Behav. Brain Sci. 12:189-250,'89). We examined the agonist burst recorded from a wrist muscle (ECRL) of human subjects (n = 7) when they performed rapid step-tracking movements under different load conditions. In each load condition, we compared the peak of the burst and its duration (at 25% of peak activity) for different amplitude movements (5 and 25 deg changes in the radial direction). The agonist burst for movements performed with a lightweight manipulandum was largely modulated in height (ave. change = 117%) and not duration (ave. change = 11%). In contrast, when subjects performed movements against elastic loads using a heavier manipulandum, modulation of burst duration became more prominent (ave. change = 63%) and modulation of burst height began to saturate (ave. change = 35%). In fact, the average duration of the agonist burst in one subject was as short as 51 msec for movements made with the lightweight manipulandum and as long as 211 msec for movements made in the loaded condition. Our results indicate that the force requirements of a task have a significant influence on whether the agonist burst displays height and/or duration modulation. Support: VA Med. Res. Service.

445.22

CONTRASTING KINEMATIC AND FORCE PATTERNS IN LIFTING FREE OBJECTS OF DIFFERENT WEIGHTS. P.L. Weir and C.L. MacKenzie. Dept. of Kinesiology, University of Waterloo, Waterloo Canada N2L 3G1.

Reaching to grasp an object requires that the arm and hand be transported to the desired location, and that functionally effective forces be applied to the object. The kinematics of the transport and grasp components until object lift have been studied extensively to identify the relationship between the two components (Jeannerod, 1981, 1984; Marteniuk et al., 1987; Wing et al., 1986). Quite independently, the force patterns of the pinch grip after object lift have been studied as a separate parameter underlying motor control processes (Cole and Abbs, 1988; Johansson and Westling, 1984, 1987, 1988; Winstein and Abbs, 1989). Our purpose was to measure and compare kinematic and grip force patterns prior to and after the application of functionally effective forces in a grasp, lift and replace task. Trials were blocked for three dowels varying in weight (66,155,423 grams) but not visual appearance. The dowel was instrumented with strain gauges which measured the vertical lifting force, and the horizontal gripping forces of the thumb and index finger. Analyses of markers placed on the thumb and index finger (grasp component), and wrist (transport component) revealed that differences in the kinematic patterns between hand lift and dowel lift reflected the application of forces after dowel contact. Temporal and kinematic analyses up to dowel contact revealed no differences; however, the time spent in contact with the dowel (prior to lifting), increased significantly as object weight increased. In addition, lifting and replacement forces varied systematically reflecting the need for stability during these two phases of movement. These results suggest a phase dependent coupling between the application of functionally effective forces and kinematic patterns. (Supported by NSERC)

445.23

ANTICIPATORY POSTURAL ADJUSTMENTS UNDER TWO TYPES OF MULTIJOINT ARM MOVEMENTS. • C.C.Bassile, C.M.Bock*, T.Kaminski*, J.R.Higgins*. Teachers College, Columbia University, New York, NY 10027.

Anticipatory postural adjustments (APA's) made prior to the initiation of multijoint arm movements must account for a variety of joint torques for smooth movement and successful goal accomplishment. This investigation demonstrates a relationship between multijoint arm movements and postural control. Two types of sagittal plane pointing movements, reaches (shoulder and elbow moving in the same direction) and whips (shoulder and elbow moving in opposite directions), were compared during three different combinations of shoulder and elbow displacements for three standing subjects (Ss). Kinematic analysis revealed the whips to have longer movement times ($\bar{X}$ MT= 513.7 ms) and larger peak velocities ($\bar{X}$ PV= 2.90 m/s) than the reaches ($\bar{X}$ MT= 366.7 ms, $\bar{X}$ PV= 1.88 m/s). The APA latencies (time from change in shank acceleration to the initiation of wrist movement) were also different for the two types of movement. Ss appeared to time their upper limb movement with a peak in shank acceleration, suggesting an efficient relationship between body COM displacement and movement initiation. Comparisons of body COM displacement to APA and wrist movement were also analyzed.

It appears that the anticipatory postural adjustment varies with movement type, displacement and amount of force generated by the upper limb movement. The task constraints implicate differential control strategies which incorporate both the limb movement and the postural component.

445.24

DISCRETE AND CONTINUOUS PROCESSES IN THE PROGRAMMING OF EXTENT AND DIRECTION IN MULTIJOINT ARM MOVEMENTS. M.Favilla*, J.Gordon, M.F. Ghilardi*, and C. Ghez. Ctr. for Neurobiol. & Behav., Prog. in Phys. Ther., Columbia Univ. and NYS Psych. Inst., New York, NY 10032.

We have previously reported that when subjects aim impulses of isometric force to targets of unpredictable amplitude and direction in 2 dimensional space, these response variables are specified in parallel and programmed by distinct mechanisms. Amplitude is programmed as a continuous variable, direction as a discrete variable. We now determine whether similar distinctions apply to multijoint limb movements and assess the time needed to program these response features.

Subjects moved a hand–held cursor on a digitizing tablet from a common start point; targets and a screen cursor were displayed on a computer monitor. A timed response paradigm was used: movements were initiated in synchrony with the last of a series of regular tones and targets appeared at unpredictable times before the last tone. Within trial blocks, targets were at either of two distances and in either of two possible directions, target separations ranging from 15° to 150° on either side of the midline.

With widely separated (>60°) targets, default responses (<100 ms after target presentation) were directed towards one or the other target, while for narrow separations they were directed towards the center. In contrast, extents were always clustered near the center of the range of required distances. As processing time increased (onset from 100 - 450 ms after target), the proportion of wrong direction responses decreased progressively, while the amplitudes and directions of initially biased responses were gradually adjusted. The processes responsible for the programming of movement direction therefore differ when alternative directions are widely separated or close together. Direction is processed categorically when directions are widely separated and as a continuous variable, like amplitude, when they are close together (Supported by NS 22715).

445.25

DEAFFERENTED SUBJECTS FAIL TO COMPENSATE FOR WORKSPACE ANISOTROPIES IN 2-DIMENSIONAL ARM MOVEMENTS. J. Gordon, M.F. Ghilardi*, and C.Ghez. Prog. in Phys. Ther., Ctr. for Neurobiol. & Behav., Columbia Univ. and NYS Psych. Inst., New York, NY 10032.

We have previously shown that patients with large fiber sensory neuropathies make large errors in programming both the extent and direction of aimed arm movements. These errors manifest as increases in variability and as biases in movement direction and extent. The purpose of this study was to determine the source of the biases. Two deafferented patients and four normal controls moved a hand-held cursor from a central starting position to targets in 24 directions on a digitizing tablet. Target and cursor positions were displayed on a computer screen, and vision of the hand and arm was blocked. The screen cursor was blanked during movement, to prevent visual corrections.

In normals, peak acceleration of the hand varies systematically with movement direction. This anisotropy reflects differences in the inertia being moved. Mean peak acceleration is twice as high in directions in which inertia is lowest. Normal subjects are able to compensate for these inertial differences by varying movement time, so that errors in extent show only a residual dependence on direction. The deafferented subjects also show a strong dependence of peak acceleration on movement direction, but do not compensate: the errors in extent show the same relative dependence on direction as the variations in peak acceleration. Systematic biases in both initial and terminal direction are also present in normals but are not as consistent across subjects. These biases produce a clustering of responses in certain directions and are markedly increased in deafferented subjects. Both extent and direction biases in patients are substantially reduced by prior vision of the limb. We propose that the lack of compensation for directional anisotropies in deafferented patients results from an inadequate internal model of the mechanical properties of the limb. (Supported by NS 22715)

445.26

INFLUENCE OF FLEXION AND EXTENSION FATIGUE ON SPEED OF MOVEMENT. N.J. Lambert, S. Peschke*, J. Draguns* and T. Hortobágyi, *Dept. of Sport Sciences/Biology, Univ. of Denver*, Denver, CO 80208

The influence of flexion (FLEX) and extension (EXT) fatigue on forearm flexion maximum speed of movement (SOM) was examined in 10 collegiate women. Ten SOM trials were performed pre and post to 3 bouts of dynamic fatigue at 60% of maximum FLEX or EXT strength, on each of 2 test days. Surface EMG burst patterns were recorded from mm. biceps and triceps brachii during SOM. Time to target increased following FLEX (11.5%, $p<.05$), but remained unaltered following EXT fatigue. Duration of the biceps first EMG burst lengthened following FLEX (13%) and EXT (8%) fatigue ($p<.05$). The biceps to triceps burst latency also lengthened following FLEX (23%) and EXT (18.5%) fatigue ($p<.05$). FLEX fatigue decreased the peak averaged EMG amplitude of the triceps burst (11%) and EXT decreased the amplitude of the biceps (22%) and triceps (12%) burst. The longer burst duration of the biceps did not compensate for the FLEX fatigue and resulted in a lengthened time to target. Following FLEX fatigue, the decreased amplitude of the triceps reflected a reduction of force to stop the slowed movement. Following EXT fatigue, reduced amplitude of the triceps did not result in the expected decreased time to target. A reduced amplitude of the non-fatigued biceps may have delimited the limb SOM. Thus, a factor other than the triceps counteractive force inhibited the biceps and restricted the flexion SOM following EXT fatigue.

446.1

A 40Hz Rhythm in Isolated Human Cortical Slices From Epileptics. RA Palovcik, SA Reid, and JC Principe. Univ. Florida, Depts. of Neurosurgery and Electrical Engineering and V A Medical Center. Gainesville, FL.

A spontaneous 40Hz rhythm was recorded from human cortical slices removed during surgery for focal epilepsy. Signals were recorded extracellularly and subjected to a fast Fourier transform with a high pass filter. There was a consistent pattern of energy present in the 35-70Hz range. Previous activity in this range has been measured in EEG only in deeper structures. The activity was also subjected to a nonlinear dynamical analysis. Correlation dimensions were calculated and found to saturate at 6.7. The system could therefore be described by a low dimensionality deterministic chaotic attractor. This rhythm may represent repetitive activity in a two-neuron cortical feedback loop. Since it occurs in isolated cortical slices, it does not require connections with the thalamus or deeper structures and therefore does not represent activity in a thalamo-cortical relay circuit. All slices removed from epileptic foci also exhibited spontaneous synchronous seizure-like discharges during which this 40Hz rhythm could be detected.

446.2

MUSIC AS A TOOL IN ANALYSIS OF INTERSPIKE INTERVAL CODES FOR LANGUAGE AND MEMORY IN HUMAN TEMPORAL LOBE. D.F. Cawthon, J. Rahn*, G.A. Ojemann, E. Lettich*, D.F. Kalk*, and D.B. Percival*. Dept. Neurosurg.,School of Music, and Appl.Physics Lab., Univ. of Washington, Seattle, WA 98195.

With informed consent and under institutional rules, we have commonly found modulation of firing frequency for normal human temporal lobe neurons by language and memory tasks, at nonessential sites during epilepsy surgery. We had occasionally noted nearly repeating sequences of single cell (ISIs) or intercell interspike intervals (ICISIs) in multiunit recordings, so we adapted a "stereotrode" (Cawthon *et al. Soc Neurosci Abstr* 1989; **15**: 302) to separate individual cells from two multiunit records. Using the LISP Kernel compositional environment (Rahn, J. *et al. Musicus* **1**(2): in press, 1990), we musically transformed the timestamps for firings of individual cells in three ways, while slowing playback X10 from realtime to hear rapid sequences. The first transformation gave each cell a separate pitch and instrument, each cell's note playing till its next note. This allowed detection of repeating single and multicell sequences, despite variation in tempo of rhythm and "melody." The second method used the reciprocals of each of a single cell's (instrument's) sequence of ISIs, scaling the resulting pitches into the 20 to 10,000 Hz range. This arrangement allowed identification of single cell ISI patterns, whether fixed or varying in tempo, better than of ICISIs. The third method applied the second to all sequential intervals, whether ISIs or ICISIs, to detect coding by ICISIs more directly, as previously reported in patients but apparently without cell isolation (Bechtereva, N. *et al. Brain Lang* 1979; **7**: 145-63), and in monkeys but without the ability to detect repeating sequences of differing tempo (Abeles, M. and Gerstein, G. *J Neurophysiol* 1988; **60**(3): 909-24). Samples of these encodings will be played and other mathematical music techniques shown for detecting more complex patterns. (Supported by NIH Grants NS21724, NS20482, & NS17111 and a Horbach Award.)

446.3

THE CORTICAL CONSENSUS: A DISTRIBUTED DARWINIAN MODEL FOR RECOGNITION AND DECISION-TO-ACT. WILLIAM H. CALVIN. Univ. of Washington, NJ-15, Seattle 98195.

Small specialized regions of cerebral cortex do exist but they are seldom essential. This is analogous to committees, which can function without all members present, where each expert member also sits on other unrelated committees, and committee size can change. MARCEL KINSBOURNE suggests that "*When wide areas of the [cortex] are involved in one mental operation... [they] can be used either for a wide-ranging but shallow encoding, or for a single but difficult mental operation.*"

But by what neural mechanism are "committee decisions" reached? Here I propose a model that builds on population-shaping known from darwinism, especially such runaway success as in speciation and the immune response. The darwinian analogies are clearest when serial-order is involved: "get set" for hammering or throwing requires the same attention to proper ordering as does the genome's DNA sequence or the antibody's amino-acid sequence. Because ballistic movements are too brief for feedback to effectively guide them, one needs serial-buffer-like neural machinery comparable to the roll for a player piano, instead holding the activation patterns of the many different muscles of hand and arm. For accurate throwing, one requires many such arrangements in tandem (a "chorus of player pianos") to reduce timing jitter. In *The Cerebral Symphony*, I discussed the spare-time applications of such serial machinery for shaping up strings of words; the "good-enough-to-speak" decision might occur when a population of randomly-varied candidate strings had (via differential reproduction of those rated highest by episodic memories) evolved to near-clones of one another (a "Darwin Machine"). Here I suggest that *consensus also underlies higher-order recognition* and that the many planning tracks greatly augment the associative memory needed for forming new categories, e.g., many different sequencer populations coming up with a similar sequence ("apples-oranges-bananas") constituting the recognition of a new category ("fruit"). Sequencing might play little role subsequently, only *acting as scaffolding during concept formation.*

446.4

TEMPORAL CONSTRAINTS IN SYNCHRONIZATION OF MOTOR RESPONSES TO A REGULAR SEQUENCE OF STIMULI. E.Pöppel, U. Müller* and J.Mates*. Inst. med.Psychol., Munich Univ., FR Germany and Inst.Physiol. Czechoslovak Acad. Sci., Prague, Czechoslovakia.

"What is synchronized?" is the basic question in sensorimotor synchronization. We suggest that synchronization is given if the temporal central availability (TCA) of a sensory stimulus falls into the same time window as the predicted TCA of the feedback of a motor response (Pöppel et al. Naturwiss. 77, 1990, p. 89). In order to investigate this problem we have chosen a paradigm where the subject has to follow a regular sequence of auditory (or visual) stimuli with motor responses. Normally, the subject's "tapping" anticipates the auditory stimuli by approx. 30 ms. Here we report that the interstimulus interval (ISI) determines the response mode. Anticipation is observed only for ISIs up to 2 or 3 seconds. For longer ISIs subjects have to react to the stimuli; they can no longer be anticipated accurately. These results are interpreted on the basis of a temporal integration process whose capacity is limited to approx. 3 seconds (e.g., Pöppel: "Time Perception" Encycl. Neurosci., p. 1215, 1987).
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446.5

A GENERAL FRAMEWORK FOR DEVELOPING THEORY IN NEUROSCIENCE, Coleman D. Clarke, Jr., and Gary Aston-Jones[1], Putman Brain Research Project, 4525 Wasatch Blvd., Suite. 310, Salt Lake City, Utah 84124; [1]Div. Behav. Neurobiol., Dept. Mental Health Sciences, Hahnemann University, Philadelphia, PA 19102

Some examples are given of the logical status of theory in physics and biology, and of its important role in helping to advance these sciences at a rapid rate. The relationship between experimental data, principles derived from the data, and theory is delineated. It is proposed that the kind of theory needed, and, in fact, attainable in neuroscience will be an abstract logical calculus (like the quantum mechanics calculus) which will make a synthesis of data and principles, so as to explain in causal terms the overall aspects of brain operation. A brief outline of such a theory will be developed using such useful principles as found in Hebb's Postulate, cell assemblies, network theory, parallel distributed processing, and chaos theory. This framework will make use of a special theory of games, as well as heuristics including a neural conditioned reflex principle, random search and relative dominance among neural loops, and the formation of a global steady state condition in the CNS. Specific examples of possible neural substrates for some of these constructs (e.g., locus coeruleus and reticular thalamic nucleus as random search generators) will be proposed. The role such a theory might play in unifying data and principles in neuroscience will be discussed.
Supported by the Mildred Andrews Fund.

446.6

DESCENDING INTERNEURONS IN THE SPINAL NETWORK OF THE GOLDFISH MAUTHNER (M-) CELL ELECTROTONICALLY EXCITE PRIMARY MOTONEURONS. J.R. Fetcho. Dept. Physiol., SUNY at Buffalo, NY 14214.

Morphological evidence suggests that descending interneurons excited by the M-cell terminate on many motoneurons, including the large primary ones known to play an important role in escapes. To examine the synaptic connections between descending interneurons and primary motoneurons, I recorded intracellularly, simultaneously from a M-axon, a descending interneuron and a primary motoneuron (N=5). Firing the M-cell led to firing of the interneurons and produced a multicomponent EPSP in primary motoneurons. Direct activation of an interneuron produced a fast EPSP in the motoneuron that was very similar to, but smaller than, a component of the EPSP produced by M-cell firing. The latencies of the interneuron-motoneuron connections were less than .2 msec, and EPSPs followed stimulation of the interneuron at more than 30Hz. The short latency and fatigue resistance indicate that the interneurons electrotonically excite primary motoneurons. However, the data do not rule out the possibility, suggested by indirect evidence, that the interneurons also produce a late chemical input to motoneurons. Thus, primary motoneurons are excited by a combination of electrotonic inputs from descending interneurons and a previously described direct M-cell input.(Support: NIH NS07593)

446.7

ANALYSES OF MULTIPLE MOTOR PATTERNS IN CHICKS. A. Bekoff. EPO Biology Dept., University of Colorado, Boulder, CO 80309-0334.

Chicks produce a wide variety of behaviors involving rhythmic leg movements. They range from very fast (eg, foot shaking) to very slow (eg, hatching). In addition, they include behaviors with alternating (walking, swimming), synchronous (hatching, hopping) and unilateral (foot shaking, head scratching) leg movements.

Using EMG recordings, we have characterized the leg motor patterns involved in 10 behaviors. Previously, we have focussed on one behavior (eg, Smith, M.B. et al., 1987, Soc. Neurosci. Abstr. 13:355; Bradley, N.S. & Bekoff, A. 1987, Soc. Neurosci. Abstr. 13:1504) or comparisons among two to three behaviors (eg, Bekoff, A. et al., 1987, Soc. Neurosci. Abstr. 13:1541; Johnston, R.M. & Bekoff, A., 1989, Soc. Neurosci. Abstr. 15:1044). The current study examines the range of variation among a larger and more diverse group of motor patterns.

EMG recordings were made from 6 leg muscles in 0- to 8-day old chicks. Recordings were digitized and cycle period, burst duration, phase, latency and interburst interval measured. Ten cycles from each of 10 chicks were typically used to analyze each behavior.

Although parameters such as cycle period or burst duration can vary significantly among motor patterns, all have some features in common. For example, they share a biphasic pattern in which one set of muscles (or bursts) consistently participates in an extensor synergy and a second set in a flexor synergy. Onset and duration of hip and ankle muscle bursts can vary significantly between behaviors. However, they are similar to one another within each behavior. In contrast the timing of knee muscle activity often differs from that of hip and ankle.

Supported by NIH grant NS 20310.

446.8

KINEMATICS OF JAW MOVEMENTS DURING DRINKING IN THE PIGEON. R. Bermejo and H. P. Zeigler. Biopsychology Program, Hunter College (CUNY), New York, NY 10021

In birds, as in mammals, drinking involves repetitive opening and closing movements of the jaw which vary the size of the oral aperture. However, in contrast to mammals, the morphology of the avian skull permits the movement of both maxilla and mandible. The kinematics of individual beak movements were studied during drinking in the pigeon.

Subjects were fixed in a stereotaxic device using a skull-mounted head-holder and an infrared LED was attached to each beak. Drinking was elicited by immersion of the beak in water. Movements of the upper and lower beak were individually monitored by a photosensitive movement transducer and the analog signals were digitized (12 bit A/D; resolution 1 ms) for subsequent analysis.

The displacements of individual beaks were analyzed with respect to the cyclical variations in the size of the oral aperture. Drinking in the pigeon involves a series of synchronized displacements of the upper and lower beak in the vertical plane, with the maxilla leading the mandible by 30-40 ms. In most cases, variations in mandible displacement accounted for most of the variation in gape amplitude. The data are consistent with previous behavioral and EMG studies of drinking in the pigeon.

(Supported by NSF Grant BNS88-10722 and NIMH Grant MH-08366.)

446.9

OCTOPAMINE ENHANCES THE EXCITABILITY OF CENTRAL NEURONS IN THE FLIGHT SYSTEM OF THE LOCUST. J.M.Ramirez and K.G.Pearson. Department of Physiology, University of Alberta, Edmonton, Canada.

Recent studies on the flight system of the locust have demonstrated that the biogenic amine octopamine regulates energy metabolism, enhances the contractility of flight muscles and increases the responsiveness of proprioceptors. Octopamine can also initiate and modulate centrally flight behaviour. The mechanisms and sites for the central actions of octopamine are unknown. We have examined the effects of octopamine on certain synaptic connections and on the membrane properties of identified neurons. The connections we studied were those linking tegula afferents to wing elevator motoneurons. Octopamine did not significantly alter the monosynaptic EPSPs but caused the appearance of a depolarization at a latency of 18-20ms. This second depolarization is polysynaptically caused by increased excitability of interneurons in the pathway from tegula afferents to elevator motoneurons. Increased excitability was found in some excitatory interneurons presynaptic to elevator motoneurons. Short current pulses (5-10 ms, 0.5-1nA) injected into these interneurons evoked burst like depolarizations which outlasted the pulses. The following findings suggest that these depolarizations are due to active membrane properties intrinsic to these interneurons: they were evoked in an all or nothing manner depending on the injected current and they could be terminated by short hyperpolarizing current pulses. Furthermore long depolarizing current pulses (500ms, 1-2nA) evoked in some cases rhythmic bursting activity without activating the flight rhythm generator as demonstrated by recording simultaneously from other flight neurons. Thus our findings suggest that octopamine modulates centrally the flight rhythm generator by inducing active membrane properties in a small population of flight interneurons. Similar effects of octopamine were observed in some neurons in the respiratory system.

446.10

ACTIVITY OF SPINAL NEURONS WITH DESCENDING AXONS DURING FICTIVE SCRATCH IN SPINAL TURTLES. A. Berkowitz and P.S.G. Stein. Dept. Biology, Washington Univ., St. Louis, MO 63130.

The turtle spinal cord contains sufficient neural circuitry to select and generate an appropriate form of the scratch reflex. In a low-spinal, immobilized turtle, cutaneous stimulation in the mid-body region elicits a rostral scratch motor pattern in hindlimb motor nerves; such stimulation in the pocket region elicits a pocket scratch motor pattern. We investigated the basis for scratch selection and generation by recording concurrent activity of hindlimb motor nerves and spinal neurons with axons that descend into the hindlimb enlargement.

The spinal cord was transected once just caudal to the forelimb enlargement and once within the hindlimb enlargement. Unit recordings were obtained via a microsuction electrode placed in the white matter at the caudal face of this multisegmental spinal cord preparation (Abstr.Soc.Neurosci.15:1118,'89). Unit receptive fields (RF's) were compared to a dermatome map (J.Comp. Neurol.295:515,'90) and to scratch form RF's (J.Neurophysiol.53:1501,'85). Units were identified as interneurons by the latency-jitter of responses to high-frequency peripheral nerve stimulation.

Units displayed either unisegmental or multisegmental RF's. Some of the latter units responded at all sites that evoked motor output. Within each category, some units showed phasically modulated activity correlated with scratch motor output, and others did not. Some units had large, multi-segmental RF's that were confined within the RF of only one scratch form. Many units that showed phasic modulation and had RF's including portions of rostral and pocket scratch RF's showed phasic modulation correlated with expression of each scratch form; this suggests that elements of interneuronal circuitry, and perhaps pattern-generating circuitry, are shared for the two forms. Other units had multisegmental RF's and were not phasically active; this suggests that substantial convergence of sensory inputs may occur prior to activation of pattern-generating circuitry. Supported by NSF Grant BNS-8908144 to PSGS and an NSF predoctoral fellowship to AB.

446.11

FUNCTIONAL SIGNIFICANCE OF BI-THRESHOLD FIRING OF NEURONS. D. C. Tam Division of Neuroscience, Baylor College of Medicine, Houston, TX 77030.

The threshold for firing of action potentials for some neurons occurs not only at depolarized membrane potentials but also at hyperpolarized potentials. The bi-threshold phenomena had been reported in a number of central neurons including thalamic (Jahnsen & Llinás, *J. Physiol.* 349:205-226, 1984), inferior olivary (Yarom & Llinás, *J. Neurosci.* 7:1166-1177, 1987), and hippocampal neurons (Stasheff & Wilson, *Soc. Neurosci. Abst.* 15:339, 1989). Of particular interest is the firing of action potentials at hyperpolarized potentials called "low-threshold spikes" by Yarom & Llinás and "baseline spikes" by Stasheff & Wilson, which can be elicited naturally during the after-hyperpolarization (a.h.p.) following a spike activation. The generation of low-threshold spikes is a voltage- and time-dependent process, accompanied by anomalous rectification, occurs during a prolonged membrane hyperpolarization for de-inactivation of ionic conductances. The functional significance of this bi-threshold firing phenomenon was suggested to be involved in the two different rhythms generated by a neuron as a periodic bi-stable oscillator (Rose & Hindmarsh, *Proc. R. Soc. Lond.* 225:161-193, 1985; Goldbeter & Moran, *Eur. Biophys. J.* 15:277-287, 1988). It can be shown that bi-threshold firing may also be used for multiplexing signals by switching modes of operation in signal processing. More importantly, hyperpolarization can be considered as *excitation* to a neuron rather than inhibition since under these hyperpolarized conditions the neuron actually becomes more excitable (i.e., closer to the low-threshold). This hyper-excitability of neurons may account for the reported susceptibility to epileptic kindling at non-depolarized potentials in hippocampal neurons. It also suggests that Hebbian synapses may be strengthened not only by the depolarizing post-synaptic potentials but also by hyperpolarizing post-synaptic potentials. (Supported by ONR N00014-90-J-1353)

446.12

HYPOTHERMIA INDUCES SPONTANEOUS RHYTHM DISCHARGE IN ISOLATED SYMPATHETIC GANGLIA. Karim A. Alkadhi and Lian-Ming Tian, Department of Pharmacology, College of Pharmacy, University of Houston, Houston, TX 77204-5515.

Most biological processes decrease in rate in response to a decrease in temperature. However, nervous tissues in general show an anamalous response to temperature in that they exhibit a decrease in spontaneous activity when the temperature is raised and an increase in activity when temperature is reduced. Isolated sympathetic ganglia are thought to exhibit no spontaneous activity unless transmitter release is induced by drugs. However, preliminary experiments in this laboratory showed that isolated sympathetic ganglia from various mammalian species fire spontaneous, often rhythmic discharge when exposed to low temperatures. Ganglia were placed in temperature-controlled oxygenated Locke solution with their rostral ends aspirated into capillary suction electrodes. Long-lasting (24 hrs), large spontaneous potentials, often occurring in singles or bursts at regular intervals, appeared with rabbit superior cervical ganglion (SCG) or dog lumbar ganglion when bath temperature was kept between 15-30°C. When the temperature is reduced below 15°C or raised above 30°C the discharge decreased in frequency and finally stopped. No discharge was seen with rat SCG at any temperature. The frequency and amplitude of potential and pattern of rhythm varied from ganglion to ganglion and was blocked by ganglion blocking agents. The presence of rhythm in these ganglia suggest the existance of an intrinsic pattern-generating mechanism which might involve polysynaptic networking.

446.13

CERTAIN BRAIN STEM LESIONS FAIL TO AFFECT CORTICALLY DRIVEN AUGMENTATION AND INHIBITION OF MUSCLE ACTIVITY IN THE RAT: D. ASDOURIAN, S.I. LENTZ, AND S. LOOK, DEPARTMENT OF PSYCHOLOGY, WAYNE STATE UNIVERSITY, DETROIT, MI 48202

The results reported below were obtained in rats anesthetized with pentobarbital (60 mg/kg.). When the frontal cortex (Cx) is stimulated (15 twin pulses/sec, duration 0.5 msec, interpulse interval 1.0 msec, current 0.4 - 0.8 mA) 2.0 mm from the midline with the anode at the level of bregma and the cathode 1.5 mm anterior to bregma, activity is reliably driven in the contralateral trapezius and rectus capitis (muscles of the neck and shoulder). If the Cx is stimulated bilaterally with subthreshold current (determined separately for each Cx), the effects of the two stimuli augment each other and drive activity in trapezius. No augmentation is seen in rectus capitis. If one Cx is stimulated with suprathreshold current, the contralateral activity driven in rectus capitis is completely blocked by suprathreshold stimulation of the other Cx. No blockage is seen in trapezius. These results can be duplicated by coupling Cx stimulation with stimulation of substantia nigra reticulate (SNr) suggesting that Cx - Cx and Cx - SNr interactions are mediated by the same mechanisms, and that the site(s) of the interaction is in the brainstem or spinal cord. Neither unilateral pyramidal tract lesions nor unilateral lesions bridging the medial longitudinal fasciculus (MLF) interstitial nucleus of MLF, and nucleus Darkschewitsch have any affect on the results of Cx - Cx stimulation described above, leaving unanswered, questions concerning the sites of interaction of outputs from the two cortices.

446.14

ROLE OF EXCITATORY AMINO ACIDS IN THE GENERATION AND TRANSMISSION OF RESPIRATORY DRIVE IN NEONATAL RAT. John J. Greer, Jeffrey C. Smith & Jack L. Feldman, Systems Neurobiology Laboratory, Department of Kinesiology, UCLA, Los Angeles, CA 90024-1568.

The involvement of excitatory amino acids (EAAs) in respiratory rhythmogenesis and the transmission of respiratory drive to cranial and spinal motoneurons was studied in an *in vitro* neonatal rat brainstem-spinal cord preparation. Motor nerve discharges were recorded from glossopharyngeal (IX), vagus (X), and hypoglossal (XII) nerves, and cervical (C1-C5) and thoracic (T1-T4) ventral roots. A barrier at the spinomedullary junction allowed for the addition of an EAA uptake inhibitor, DHK, or EAA receptor agonists (NMDA, kainic acid, AMPA) or antagonists (MK801, CNQX, AP4) to bathing solutions surrounding either the spinal cord or brainstem.

Inhibition of the reuptake of endogenously released EAAs by adding DHK to the brainstem bath caused a dose-dependent increase in respiratory frequency. Blocking of non-NMDA receptors at the brainstem with CNQX produced a dose-dependent decrease in respiratory frequency and amplitude of cranial motoneuron discharge, whereas the agonists, kainic acid and AMPA, caused an increase in frequency. CNQX applied to the spinal cord decreased the amplitude of all respiratory spinal motoneuron discharge. Neither blocking NMDA receptors with MK801 nor blocking AP4-sensitive receptors within the brainstem affected respiratory frequency or amplitudes of cranial motoneuron and bulbospinal respiratory premotoneuron discharge. NMDA produced a dose-dependent increase in respiratory frequency. MK801 added to the spinal cord bath caused a 10-30% decrease in the amplitude of respiratory spinal motoneuron discharge. AP4 added to the spinal cord abolished the inspiratory activity recorded from C4 and thoracic ventral roots, but caused only a slight decrease in C1-C2 ventral root discharge.

We conclude that respiratory rhythm generation is dependent on a conditional drive mediated by EAAs acting at non-NMDA receptors. The transmission of drive to cranial and spinal respiratory motoneurons is also mediated by EAAs acting at non-NMDA receptors. Activation of NMDA receptors plays only a minor role in the transmission of respiratory drive to motoneuron pools, and does not seem necessary for rhythmogenesis. The binding of AP4 to its receptor strongly inhibits the transmission of respiratory drive to phrenic and intercostal motoneuron pools, but has little affect on rhythmogenesis, or the drive transmission to cranial and accessory motor pools. Supported by NIH grants HL 40959 and HL 02204.

446.15

CLASSIFICATION OF NOISY ACTION POTENTIALS (APs) BY MEANS OF A NEURAL NETWORK EMPLOYING BACK-PROPAGATION. I. Espinosa E. and J. Quiza T.*, Cybernetics Lab., Faculty of Sciences, UNAM, México 04510.

Metal electrodes allow to simultaneously register APs from different neurons, creating the need of techniques for spike sorting. As a first step we have been implementing a method to classify noisy APs. Using a database of 64 significantly different APs as the training set we generated a network with 97 processors forming two hidden layers of 30 and 60 and an output layer with 7 neurons. The network is modifiable at will. APs consist of 128 points normalized between 0 and 1. These values go into an input buffer that receives them unchanged. For employing the back-propagation training algorithm we set the initial weights (9690) between -.5 and .5. After 35,000 iterations that took 20 min CPU in the TITAN(Ardent), with 16 MFLOPS, the total system error was less than .01. The final weights are fed to a conventional PC for classifying APs. Gaussian noise was added to original APs randomly modifying amplitude up to 40%. The network was able to classify 90% of them correctly. The next step will be to sort APs embedded in a noisy spike train.

446.16

MULTIPLEXED ADAPTATION AND TRANSMISSION IN ARTIFICIAL NEURAL NETWORKS WITH INCORPORATION OF TWO SIMULATED POTASSIUM CONDUCTANCES ALLOWS FOR ASSOCIATIVE CONDITIONING AND JUDGEMENTS OF CONTIGUITY. Jon Berner & C.D. Woody. MRRC, BRI, UCLA Hlth. Sci. Ctr., Los Angeles, CA 90024.

Features of two potassium conductances implicated in the acquisition of conditioned reflexes, the slow gK+(Ca) and gK+(A), were incorporated into a 6*6 element artificial neural network with real-time, thresholded spike transmission and additive integration of PSPs. Adaptive algorithms changed gK+(A) in proportion to the product of this current and an EPSP-induced second messenger concentration and changed gK+(Ca) as a function of a spike-induced second messenger concentration.

This network acquired two distinct representations in response to presentation of stimuli: one resembled associative conditioning (defined in terms of its sensitivity to forward pairing vs. simultaneous or backward pairing); the other was sensitive to contiguous pairings of stimuli.

The acquisition of one representation did not markedly interfere with acquisition of the other; this network may accordingly serve as an example of a system which minimizes the postulated inherent cross-talk between functionally dissimilar representations (see Minsky & Papert, 1988).

446.17

BIOLOGICALLY DERIVED SYNTHETIC NEURAL NETWORKS W.E. Faller and M.W. Luttges. Aerospace Engineering Sciences, University of Colorado, Boulder, Colorado 80309-0429.

A major difficulty in developing neural network simulations of biological data has been the absence of techniques for interweaving neurobiological data and connectionist architectures. Techniques for implementing such networks are being developed and tested in our laboratory. The present developments permit experimental neural data to be directly incorporated within connectionist architectures. The spatial distribution of cells, the physiologic operating range of each neuron and the stochastic history of the cellular spike trains were retained. Sigmoidal activation functions describe the behavior of each unit. These functions were derived directly from the cellular spike history data. Each neuron was thereby represented by a unique network unit that mimicked both the mean firing frequency and temporal dynamic range of the physiologic cell. An "analog" function that preserved both the exact spiking times and the relative spiking tendency of each neuron was substituted for each spike train. The "effective" influence of each cell is reflected in the cellular dynamic range and the stochastic temporal modulations of individual cell firing patterns. These types of networks provide a means of accurately resynthesizing biological data where various connections, cell discharge characteristics or other accessible parameters may be altered. Functional hypotheses can be developed to compare experimental and analytical issues related to the biological nervous system. Alternatively, hypotheses derived from the analyses of experimental neural data may be tested by appropriate modifications of the synthetic architecture.

446.18

Memory Properties in the 1000-Neuron Inhibitory-Feedback Network Model for Simulation of Neostriatal Circuits. C.D. Myre and D.J. Woodward. *Dept. of Cell Biology and Neuroscience, UT Southwestern, Dallas, Texas 75235.*

This laboratory has reported previously on the capability for information storage, or memory, in the states of networks of mutually-inhibitory model neurons. Continuing investigation of this memory in the 1000-Neuron Network Model (which construction was inspired by medium spiny neurons) has revealed several new properties showing how state maintenance and state switching occur. The studies described here were done using the XNET Simulation System (Neurosci. Abstracts 1989, #416.18). The calculations were performed on a Sun SPARCstation 370 computer. The 1000-Neuron Network Model is made up of 1000 model neurons with realistic electrical properties, with parameters set by the user. Each neuron is connected to a variable, spherical domain of nearby neurons by inhibitory synapses. Excitation can occur via excitatory synapses which are controlled by the user, and Gaussian "noise" (representing lumped, random synaptic activity) may also provide excitatory and inhibitory effects. The parameter settings in initial experiments were such that, after decay of inhibition and potassium conductance for a neuron, the membrane potential reached threshold, and the neuron fired. When inhibitory feedback was added to the eight nearest neighbors of each neuron, the neurons entered and maintained states in which they either fired repeatedly (the "on" state), or did not fire at all (the "off" state). The results showed: 1. that the maintenance of state (i.e., memory) requires setting parameters so that IPSP's are relatively long-lasting; 2. even though states could apparently be maintained indefinitely, state-switching required only a few user-supplied excitatory impulses; and 3. state changes with local reciprocal effects did not necessarily propagate globally, despite the high connectivity. Also, powerful global noise can induce spontaneous switching, which facilitates evolution to a system ground state. This modeling suggests that the structural organization of neostriatum could support short-term memory. *Support from the Texas Adv. Res. Prog., Biol. Humanics Found., MH44337, DA02338, and AFOSR 90-146.*

446.19

WITHDRAWN

ASSOCIATION CORTEX AND THALAMOCORTICAL RELATIONSHIPS

447.1

NICOTINIC AND MUSCARINIC DEPOLARIZING RESPONSES OF THALAMOCORTICAL CELLS TO STIMULATION OF BRAINSTEM CHOLINERGIC NUCLEI. R. Curró Dossi, D. Paré and M. Steriade. Lab. Neurophysiol., Sch. Med., Univ. Laval, Quebec, Canada, GlK 7P4.

Preliminary data showed that a long-latency (1-1.5 s) depolarization, lasting for 1.5-2 s, is elicited in lateral geniculate cells by stimulation of the peribrachial (PB) area (Steriade and Deschênes, l988). We have further investigated the mechanisms of brainstem-induced depolarizing events in 85 thalamocortical cells recorded intracellularly from various nuclei in urethane-anesthetized cats. Stimulation of PB or laterodorsal tegmental (LDT) nuclei induced two types of depolarizing responses that resisted monoamine depletion by reserpine treatment. (a) The first component had a latency ranging from 20 to 140 ms, lasted for 1.7 ± 0.12 s, was associated with 20-30% increase in membrane conductance, and was blocked by mecamylamine. (b) The second component had a latency of 1.4 ± 0.3 s, lasted for 5 to 30 s, increased under steady depolarization, often disappeared with cell hyperpolarization, was associated with 30-50% increase in apparent input resistance, and was accompanied by an EEG desynchronization with a similar time-course. After scopolamine treatment, the long-lasting depolarization disappeared; the surviving short-lasting depolarization was abolished by subsequent administration of mecamylamine. These data demonstrate that, following the initial nicotinic depolarization, PB/LDT stimulation elicits a long-lasting muscarinic depolarization that is probably involved in cortical EEG activation. Supported by MRC grant MT-3689.

447.2

DIMENSIONAL ANALYSIS OF EVENT-RELATED POTENTIALS. M.Molnár*, J.Desmedt*, J.E.Skinner. Neurophysiology Sect. Neurology Dept., Baylor College of Medicine, Houston, TX 77030.

Temporal alterations in the correlation dimension (D2) were calculated from event-related potentials (ERPs) evoked in humans. The ERPs (1,000-Hz A/D) were evoked by weak pulses, delivered separately to one of two fingers, while the subject was either reading a novel (control) or detecting and counting infrequent stimuli to one of the fingers (target). The point-D2 algorithm of Skinner and associates was used to analyze 15,000-point epochs made of 15 linked trials. Fifteen-trial averages of the point-D2 were made following this analysis. In the parietal primary cortex target stimuli evoked a D2 DECREASE of 1.0 dimensions (from a mean and standard deviation of 5.6 ± .15), in parallel with the P300 ERP-component (P<.001). In the frontal association cortex an earlier D2 decrease of 0.7 dimensions occurred (from 5.5 ± .10), in parallel with the N140 component of the ERP (P<.001); this initial decrease was followed by a D2 INCREASE of 0.7 dimensions, peaking at 400 msec (P<.001) and returning to baseline by 500 msec. Although control stimuli evoked some large ERP components, there was no significant change in the point-D2. These data suggest that specific processes of different dimensional complexities occur in the primary and association cortices during analysis of relevant information.

447.3

CYTOARCHITECTONIC REMAPPING OF AREAS 9 AND 46 IN THE HUMAN PREFRONTAL CORTEX. G. Rajkowska and P. S. Goldman-Rakic. Section. of Neuroanat.,Yale Univ. Sch. of Med., New Haven, CT 06510.

The location of human prefrontal areas varies between Brodmann's widely used map (1909) and the more detailed analyses of von Economo & Koskinas (1925) and Sarkissov et al. (1955). The need for a uniform parcellation of the human brain stimulated us to reinvestigate the cytoarchitecture of the prefrontal cortex beginning with areas 9 and 46. We analyzed Nissl-stained, celloidin-embedded sections of the left hemisphere from 8 human brains. Data on cell size, cell packing density per layer, and layer thickness supplemented light microscopic observations.

Among prefrontal areas, area 9 is distinguished by its large pyramids in sublayers IIIc and Va and by distinct lamination. Three subdivisions can be recognized based on size of pyramids, distinctness of radial striations and cortical thickness. Using these criteria, area 9 was located on the anterior and middle portions of the first and superior part of the second frontal convolution extending to the superior frontal sulcus in all cases examined. Area 46 can be distinguished from surrounding areas by the more uniform size of its pyramids, a generally higher cell packing density, and a wider and more regular layer IV having distinct borders with adjacent layers. Area 46 was located in the middle of the second frontal convolution. Although its inferior border was consistently found in the fundus of the inferior frontal sulcus, it was never observed on the ventral convexity as represented in Brodmann's map. Variability among cases was greater in area 46 than in area 9 and appeared due to individual differences in the number and configuration of gyri in the second frontal convolution.

The present localization of areas 9 and 46 corresponds more to the maps of von Economo and Sarkissov than to that of Brodmann. Our preliminary findings suggest that the surface area of area 46 may vary considerably among individuals and may underlie their individual differences in certain visuo-spatial capacities. Supported by MH44866.

447.4

THE ORGANIZATION OF PROJECTIONS FROM THE MEDIODORSAL NUCLEUS OF THE THALAMUS TO ORBITAL AND MEDIAL PREFRONTAL CORTEX IN THE MONKEY. J. P. Ray and J. L. Price, Dept. of Anat. & Neuro., Washington Univ. Sch. Med., St. Louis, MO 63110.

The organization of the mediodorsal thalamic nucleus (MD) of the cynomologous monkey was examined with retrograde and anterograde axonal tracers, and an analysis of cyto- and myeloarchitecture. Our results verify the conclusions of previous studies (e.g., Goldman-Rakic and Porrino, '85), but also demonstrate additional features of the organization of MD. In addition to the magnocellular division of MD (MDm) and its projection to orbital cortex, we recognize a separate caudodorsal division as the source of projections to medial prefrontal cortex. Projections of MD were studied by placing small injections of fluorescent retrograde tracers restricted to single cytoarchitectonic areas in the medial or orbital prefrontal cortex, or the agranular insula. After injections into orbital cortex (areas 11, 13 and 13b), labeled cells are found in MDm, which largely corresponds to the densely myelinated MD pars fibrosa in myelin preparations. In contrast, injections of retrograde tracers into medial prefrontal cortex (areas 32, 24) label cells in MD which are beyond the dorsal and caudal limits of MDm; cells projecting to area 32 are found at the dorsal edge of MD, along its entire rostrocaudal extent, while cells projecting to area 24 are concentrated in the caudomedial portion of the nucleus. These parts of MD partially correspond to MD pars densocellularis of Olszewski ('52), but more precisely correspond to a poorly myelinated portion of MD referred to as pars caudalis in human material (Hassler, '59). Cells projecting to the agranular insula are limited to the medial edge of MD, a region which is poorly myelinated and may correspond to a medial extension of the caudodorsal division of MD. Anterograde label from prefrontal cortical regions (areas 32, 13, and agranular insula) reciprocate this pattern. Support; NIH DC00093-20.

447.5

SYNAPTIC ORGANIZATION OF CORTICAL EFFERENTS AND GABA-CONTAINING SYNAPTIC ELEMENTS IN THE MEDIODORSAL THALAMIC NUCLEUS OF THE MONKEY. M. L. Schwartz, J. J. Dekker* and P. Goldman-Rakic. Sect. of Neuroanatomy, Yale Univ. Sch. of Medicine, New Haven, CT 06510

We examined the synaptic organization of cortical inputs and GABA containing synaptic circuits in the monkey mediodorsal nucleus (MD). To examine cortical inputs, we injected the prefrontal cortex of two rhesus monkeys with ^{3}H-leucine and proline and analysed the distribution and morphology of radiolabeled terminals in the MD. The synaptic organization of GABA-containing elements was examined in four additional monkeys using GABA immunohistochemistry (Incstar).

Quantitative analysis of the density of silver grains over different tissue compartments in the monkeys receiving cortical injections of ^{3}H-leucine and proline, revealed a positive labeling index for two terminal classes. Relative grain density was greatest for *small terminals*, with round synaptic vesicles in the extraglomerular neuropil. In addition, a number of *large terminals*, with round vesicles were labeled within glomeruli. These terminals were presynaptic to the central dendrite, as well as to presynaptic dendrites of the glomerulus. Both classes of terminal formed asymmetric synaptic densities.

In GABA immunoreacted tissue the majority of reactive presynaptic profiles were found within the glomeruli. Nearly all of these could be classified as presynaptic dendrites (PSDs) and formed symmetric synaptic contacts with the central dendrite. Two classes of presynaptic elements were also labeled in the extraglomerular neuropil, although their numbers were more limited than that of the PSDs in glomeruli. One class consisted of extraglomerular PSDs which formed symmetric synaptic contacts with unlabeled dendrites. A small number of axon terminals were also immunoreactive. These were generally small and were densely packed with vesicles. Although these were most frequently found to contact unlabeled dendrites, a small number were presynaptic to neuronal cell bodies.

These results suggest that cortical input to the MD arrives via two distinctive synaptic pathways, one ending extraglomerularly and the other acting on GABAergic PSDs and relay cell dendrites within glomeruli. Supported by BNS 8617585

447.6

PREFRONTAL CONNECTIONS OF MEDIAL PREMOTOR AREAS IN THE RHESUS MONKEY J.F. Bates & P.S. Goldman-Rakic. Sect. Neuroanatomy, Yale Univ. Sch. Med., New Haven, CT 06510

Higher-order motor areas, implicated in initiation and execution of skilled movements, have been localized on the medial surface of the frontal lobe in both monkey and human. SMA, (supplementary motor area), or the medial aspect of area 6, has connections with primary motor cortex as well as somatosensory cortex and the spinal cord. Each is thought to have a somatotopic representation of the body surface. Additional premotor areas have been suggested in cingulate cortex (e.g. He et al., 1989). We have investigated the connections of prefrontal cortex with the SMA and cingulate cortex in the rhesus monkey, using small injections of wheat germ agglutinin-conjugated horseradish peroxidase (WGA-HRP) in each of several distinct areas of prefrontal cortex (Walker, 1940).

Analysis of WGA-HRP - labelled cells and terminals reveals that Walker's areas 8a, 9, 12, and 11/13 have connections with rostral SMA . All of these areas are also connected with the anterior third of the cingulate gyrus and in some cases with the ventral bank of the cingulate sulcus. One additional area, the ventral rim of the principal suclus (46) is also connected with cingulate cortex. Another subset of prefrontal areas (8a, 9, 11/13, dorsal rim 46, and 10) is connected with the middle third of cingulate cortex, including the ventral bank of the cingulate sulcus in some cases. A few areas (8a, dorsal rim 46, 10) were connected with the posterior third of the cingulate gyrus. No connections were found between area 45 and medial prefrontal cortex. In summary, all of the prefrontal areas examined (with the exception of area 45) project onto the SMA and/or cingulate cortex in what appears to be a topographic manner. These data could provide transcortical pathways by which prefrontal cortex influences the motor cortex to issue a particular command. The prefrontal-premotor pathway would thus complement the striato-nigral-thalamo-cortical path in control of movement.

447.7

FRONTO-TEMPORAL CONNECTIONS IN THE RHESUS MONKEY. C.L. Barnes and D.N. Pandya. Bedford Veterans Hospital, Bedford, MA 01730 and Depts. of Anatomy and Neurology, Boston Univ. Sch. of Med., Boston, MA 02118.

The frontal lobe connections from the auditory association areas of the Superior Temporal Gyrus (STG) have been shown to be organized according to architectonic specialization such that the less differentiated areas of temporal polar cortex project to the less differentiated areas of medial and orbital frontal lobe cortices. The highly differentiated areas of caudal STG project to the more differentiated areas of caudal prefrontal cortices. Areas having intermediate architectonic features within STG are preferentially connected with prefrontal lobe regions characterized by similar architectonic characteristics (Petrides and Pandya, 1989). In order to study the reciprocal relationship of frontal lobe projections to the auditory association regions, Fluorescent Retrograde Tracers were injected into the different subdivisions of STG. The results show that less differentiated areas of STG (Pro, Ts1, Ts2) receive preferential projections from prefrontal lobe areas with similar architectonic features of orbital and medial prefrontal cortex (Pro, 13, 14, 10, 25, 32), whereas highly differentiated areas of caudal STG (PaAlt, Tpt) receive preferential projections from caudal prefrontal regions with similar architectonic features (46, 8). The middle areas of STG (Ts3) with intermediate architectonic characteristics are connected with areas 46, 12 and 11 as well as to nearby regions, areas 25, 32 and 8. The more rostral areas of STG receive projections from widespread areas of prefrontal cortex whereas caudal STG receives projections from restricted areas. These observations suggest that fronto-temporal connections are reciprocal to temporo-frontal connections and are organized according to architectonic characteristics of the regions. (Supported by Veterans Administration, ENRM VA Hospital, Bedford, MA, NIH Grant #16841).

447.8

THE COURSE OF THE TEMPOROPONTINE FIBER SYSTEM IN RHESUS MONKEY. Jeremy D. Schmahmann and Deepak N. Pandya. Massachusetts General Hospital and Harvard Medical School, Boston, MA 02114; ENRM Veterans Hospital, Bedford, MA 01730, and Boston University School of Medicine, Boston, MA 02118. The temporal lobe projections to the pons have been the subject of debate for many years (e.g. Turck, 1851; Dejerine, '03; Nyby and Jansen, '51; Brodal, '78). We have previously described the temporopontine projections from the superior temporal sulcus (STS) (Schmahmann and Pandya, '89). In the present study we have analyzed the course of the temporopontine fiber system from the STS, as well as from the supratemporal plane and the superior temporal gyrus in the rhesus monkey using the technique of autoradiography. From the injection site, labelled fibers could be traced into the white matter along with the cortical and other subcortical fibers. From rostral injection sites the fibers coursed caudalward, and after more caudal injections the labelled fibers progressed rostrally. At the level of the mid-portion of the lateral geniculate nucleus (LGN), a well defined fiber fascicle could be seen to separate from the other fiber bundles. This fascicle travelled medially over the dome of the LGN, above the geniculocalcarine pathway but below the fiber bundles destined for thalamus or the colliculi. Descending medial to the LGN, the temporopontine fibers concentrated in an aggregate of curvilinear lamellae situated rostral to the medial geniculate nucleus (MGN). Fibers then descended in an oblique caudal direction, passing inferior to the MGN, and entering the cerebral peduncle in its lateral sector. They continued their descent in the lateralmost aspect of the pons and formed a triangular bundle located dorsal and lateral to the pontine nuclei. Fibers then peeled off and terminated in their respective nuclei as described previously (Schmahmann and Pandya, '89). The use of the autoradiographic technique has thus permitted a clear delineation of the temporopontine projection in the rhesus monkey. (Supported by the Veterans Administration, ENRM VA Hospital, Bedford, MA; and NIH Grant #16841).

447.9

POSTNATAL DEVELOPMENT AND SOURCES OF CHOLECYSTOKININ (CCK) IMMUNOREACTIVITY IN MONKEY PREFRONTAL CORTEX (PFC). K.M. OETH AND D.A. LEWIS. Depts. of Behavioral Neuroscience and Psychiatry, Univ. of Pittsburgh, Pittsburgh, PA 15213.

The CCK innervation of adult monkey PFC exhibits a unique laminar pattern without regional differences in density. However, neither the postnatal development nor the origins of this innervation have been examined. We used immunohistochemical techniques with an antibody which recognizes the terminal pentapeptide of CCK to characterize CCK-immunoreactive (IR) structures in PFC of monkeys (*Macaca mulatta* and *fascicularis*) aged 4 days to adult. The density of CCK-IR neurons and fibers was greatest in the youngest monkeys. In monkeys 77 days or older, neuron and fiber density substantially decreased. Regionally, neuron and fiber density was greater in ventral than in dorsal regions of the youngest monkeys but was very similar in monkeys 77 days or older. Laminar differences in density were also evident. In layers III-VI, fascicles of radial fibers were present which were very dense in the youngest monkeys. These fascicles were composed of two types of fibers: smooth, helical fibers of large caliber or thin varicose fibers. At 77 days or older, a low density of single, varicose, radial fibers was observed. With minor regional variations, all monkeys exhibited terminal fields in layers II, IV and VI. Retrograde transport techniques were utilized to investigate the origins of these terminal fields. The mediodorsal nucleus of the thalamus (MD) is known to project to layer IV of PFC. However, following injections into PFC, retrogradely labeled neurons in MD were not CCK-positive although CCK-IR neurons were present in neighboring thalamic nuclei. Further investigations of the origins of the CCK-IR terminal fields in layers IV and VI are in progress.

447.10

POSTNATAL CHANGES IN THE DOPAMINERGIC INNERVATION OF MONKEY PREFRONTAL CORTEX: A TYROSINE HYDROXYLASE IMMUNOHISTOCHEMICAL STUDY. H.W.Harris* and D.A.Lewis. Departments of Psychiatry and Behavioral Neuroscience, Univ. of Pittsburgh, Pittsburgh, PA 15213.

Dopaminergic (DA) projections to adult monkey prefrontal cortex (PFC) exhibit distinctive regional and laminar patterns of innervation. However, little is known about the development of these innervation patterns. In this study immunohistochemical techniques were used to characterize the distribution of tyrosine hydroxylase (TH)-immunoreactive (IR) axons in PFC (areas 8B, 9, 10 and 46) of monkeys (*Macaca mulatta* and *fascicularis*) aged 4 days to adult. The anti-TH antibodies used in this study have been shown to selectively label DA axons in monkey PFC (Br Res 500:313, 1989). In all regions of the youngest animals, the density of TH-IR fibers was greatest in layers I-superficial III, lowest in layers deep III-V and intermediate in layer VI. However, by 77 days of age the dorsomedial convexity (areas 9 and 8B) exhibited a marked increase in the density of labeled fibers in layers deep III-V. This apparent ingrowth of TH-IR fibers persisted into adolescence and adulthood. In contrast, the laminar distribution of labeled fibers in areas 46 (dorsolateral surface) and 10 (frontal pole) did not change during postnatal development. These results reveal regional and laminar specific developmental changes in the DA innervation of monkey PFC that account for the distinctive patterns of innervation present in the adult. These findings also suggest that there may be regional differences in the effects of DA on the maturation of PFC functions.

447.11

POSTNATAL DEVELOPMENTAL CHANGES IN PARVALBUMIN (PV) IMMUNOREACTIVE (iR) AXON TERMINALS OF BASKET AND CHANDELIER NEURONS IN MONKEY NEOCORTEX. M. Akil and D.A. Lewis. Department of Psychiatry and Behavioral Neuroscience, University of Pittsburgh, Pittsburgh, PA 15213.

Basket and chandelier cells are GABAergic inhibitory interneurons, and important regulators of pyramidal cells. We used immunohistochemical methods to determine the location of PV, a calcium binding protein, in axon terminals of these two classes of interneurons during postnatal development in monkey neocortex. We examined prefrontal (area 46), primary motor (area 4) and visual (areas 17 and 18) cortices of nine *Macaca mulatta* and *fasicularis* monkeys aged 4 days to adult. PV-IR varicosities formed pericellular baskets around unlabeled pyramidal somata in layer V of area 4 (Betz cells), in layers V and VI of area 17, and in layer V of area 18. In these regions PV-IR pericellular baskets were present as early as 4 days of age and absent in the adult. Both the time course and pattern of their disappearance were region-specific. No PV-IR baskets were seen in area 46 at any age. Chandelier neuron axon cartridges were formed by PV-IR varicosities aligned in vertical rod-like structures. PV-IR cartridges appeared later than pericellular baskets and remained present in the adult. In area 17, PV-IR cartridges were located in layers II-superficial III at all ages. In contrast, the highest density of PV-IR cartridges in areas 4, 18 and 46 shifted from the middle layers in infant monkeys to layers II-superficial III in the adult. The differential regional, laminar and developmental pattern of PV-IR axon terminals of chandelier and basket interneurons may provide insight into their function.

447.12

HETEROGENEITY OF THALAMIC CELLS PROJECTING TO LAYER I IN POSTERIOR PARIETAL CORTEX OF CAT AND MONKEY. C. Avendaño, I. Stepniewska*, E. Rausell* and F. Reinoso-Suárez. Department of Morphology, Medical School, Autónoma University, 28029 Madrid, Spain.

The thalamic neurons projecting to superficial layers of cortical areas 5 (cat and monkey) and 7 (cat) were investigated by using superficial deposits of retrograde tracers in one hemisphere, and full-depth cortical injections in homotopical locations of the contralateral one. In the cat, labeled neurons in the LP-Pul complex, and in paralaminar nuclei, were fewer in number and smaller in size in cases of superficial deposits than in cases of deep injections. In non-paralaminar portions of VL, however, there were no size differences. In the monkey, similar differences in number and size appeared in the caudal division of VL and in LP and Pul, whereas no such differences were found between neurons labeled in the oral and medial divisions of VL, and in the ventral posteroinferior nucleus. The intralaminar and midline nuclei exhibited retrogradely labeled neurons only when deep layers were injected. These findings point to the existence of a widely distributed layer I-projecting system (LIPS) of neurons which, in most nuclei, are interspersed among neurons projecting mainly to middle or deep layers. The paralaminar nuclei, which would be but a part of this system, could provide through their projections to layer I in the posterior parietal and frontal cortical regions a final path for recruiting responses and spindling activities.
Supported by Grant PB87-0130 from CICyT.

447.13

CORTICAL AND THALAMIC CONNECTIONS OF THE ORBITAL CORTEX IN THE RAT. F.Condé and F.Crépel. CNRS URA 1121, Bat.440, Université Paris-XI, 91405- Orsay, France.

In the orbital cortex (OC) of the rat, Zilles's atlas (1985) reports 3 subdivisions: the medio- ventral (MO/VO), lateral (LO) and ventro- lateral (VLO) parts of OC, from which connections are virtually unknown. The retrograde transport of fluorescent tracers diamidino yellow and true blue was used to investigate the connections of these orbital areas with the other areas of the medial frontal cortex (MFC), the postgenual cingulate (Cg) and the retrosplenial (Rs) cortices.

In contrast to the adjacent insular and prelimbic cortices, OC receives direct afferents from the central part of the ipsilateral mediodorsal nucleus of the thalamus, the anterior olfactory nucleus and the pyriform cortex. These results strongly support the hypothesis of an homology between OC in rat and OC in monkeys. From the pattern of efferents from OC to MFC, postgenual Cg and Rs, another delineation of the subdivisions of OC in two parts appears: 1) a rostral orbital area, including MO/VO, and rostral part of LO and VLO, which projects to MFC, postgenual Cg and Rs, and 2) a caudal orbital area, including the caudal part of LO and VLO, which projects only to anterior cingulate and prelimbic cortices.
supported by HFSP grant.

447.14

REACTIONS TO FAMILIAR OR NOVEL ENVIRONMENTAL CUES AI ORIENTATION BEHAVIOR OF RATS WITH UNILATERAL OR BILATERA MEDIAL AGRANULAR CORTEX (AGm) LESIONS. J.M.Vargo P.J.Best, L.D.Hall*, S.L.Spera* and J.V.Corwin. Departmer of Psychology, University of New Orleans, New Orleans, L 70148.

To examine the role of dorsomedial prefrontal cortex attentional processess, Long Evans rats were trained traverse two straight alleys for food. Subjects th received sham, unilateral AGm, or bilateral AGm lesion Postoperatively, the subjects received further trainin Then the goal boxes were changed such that they contain novel cues or contained familiar cues not previous experienced there.

Alley running latencies of the bilateral operat indicated that they noticed the unfamiliar ($p = .02$) b not familiar cue change. These results are similar to tho found with bilateral medial dorsal thalamic lesio (Stokes, K.A. & Best, P.J, Neurosci. Abs., 13:1067, 1987 Unilateral AGm operates demonstrated similar deficits attention to environmental changes in the alley.

The subjects also underwent orientation testing visual, tactile or auditory stimuli presented to each bo side. Bilateral operates did not demonstrate orientatic deficits while left AGm operates demonstrated contralater neglect (J.M. Vargo et al., Exp. Neurol., 102:199, 1988).

Even though bilateral lesions fail to produce neglec they do produce spatial context recognition deficits.

447.15

CYTOARCHITECTURE AND CORTICAL AFFERENTS OF THE MONKEY ORBITOFRONTAL CORTEX.
Morecraft, R.J., Geula, C., Schatz, C. and Mesulam, M-M., Harvard U., Boston, MA.

Orbitofrontal cortex (OFC) is a major paralimbic component of the primate brain and can be divided into agranular (periallocortical), dysgranular and granular (isocortical) sectors. The agranular and dysgranular sectors are more caudal and medial than the granular sector. HRP injections were made within the agranular-dysgranular sector of the OFC in one monkey and into the granular sector in another. Both cases contained labeled neurons within the cingulate cortex and all components of the prefrontal cortex. The case with the tracer injection in the granular OFC contained labeled neurons in the periinsular parietal operculum, high order association cortex of the superior temporal sulcus and the granular sector of other paralimbic areas such as the insula and temporopolar cortex. The case with the tracer injection in the agranular-dysgranular sector of OFC showed a different pattern. Labeling was seen in the agranular-dysgranular components of the insula and temporopolar cortex and there was less extensive labeling in the superior temporal sulcus. In the amygdala, labeling was much more intense in the case with the agranular-dysgranular injection. These observations show that the monkey OFC displays a complex pattern of connectivity which follows cytoarchitectonic lines of demarcation and which is in keeping with its behavioral affiliations.

447.16

SOME SPECIES DIFFERENCES OF THE CHOLINERGIC BASAL FOREBRAIN IN RAT AND MONKEY. Ch.R. Schatz, C. Geula, R. Morecraft and M-M. Mesulam, Harvard U., Boston, MA

We investigated NADPH-diaphorase activity and calbindin D-28k immunoreactivity (antisera generously provided by L.B. Hersh, B. Wainer, M. Celio) in the cholinergic projection neurons of the rat and monkey basal forebrain (Ch1-4) and brainstem (Ch5-6), specifically concentrating on species differences. We could confirm the nearly total overlap between NADPH-diaphorase activity and choline acetyltransferase (ChAT)-immunoreactivity in the brainstem (Ch5 and 6) in both species. In the rat, we found NADPH-diaphorase activity in up to half of the ChAT-positive cells of the vertical limb nucleus of Broca (Ch2) and in approximately 20% of ChAT-positive cells in the medial septal nucleus (Ch1), but not within the nucleus basalis (Ch4). In the monkey basal forebrain (Ch1-4), colocalization of NADPH-diaphorase activity and ChAT-immunoreactivity occurred very rarely, if at all. As reported previously by Celio and Norman (Anat. Embryol. 1985), the nucleus basalis (Ch4) neurons in the monkey contained calbindin, suggesting that ChAT and calbindin are colocalized in these neurons. No such overlap could be found in the rat. Adjacent sections stained for ChAT and calbindin revealed the absence of calbindin immunoreactivity in Ch5 and 6 in both the monkey and the rat. These findings demonstrate a major species difference in the chemoanatomical profile of the cholinergic basal forebrain in rodents and primates. The cholinergic projection cells of the brainstem did not show such species differences.

447.17

CHOLINERGIC NEURONS IN THE RAT SEPTAL COMPLEX: ULTRASTRUCTURAL CHARACTERIZATION AND SYNAPTIC RELATIONS WITH CATECHOLAMINERGIC TERMINALS.

T.A. Milner. Div. of Neurobiology, Dept. of Neurology and Neuroscience, Cornell Univ. Med. Coll., New York, NY 10021.

The ultrastructural morphology of neurons containing choline acetyltransferase (ChAT) and their relation to catecholaminergic terminals exhibiting immunoreactivity for the catecholamine synthesizing enzyme tyrosine hydroxylase (TH) were examined in the rat medial septal and diagonal band nuclei using dual immunoautoradiographic and peroxidase anti-peroxidase labeling methods to simultaneously localize antibodies from two different species. Perikarya with ChAT-immunoreactivity (ChAT-I) were large (20-30 μm), elongated and contained an abundant cytoplasm. Many of the perikarya and dendrites with ChAT-I were surrounded by glial processes. Synaptic junctions on ChAT-labeled perikarya and dendrites were both symmetric and asymmetric with 68% (135 out of 197) of the presynaptic terminals unlabeled. The remaining terminals were immunoreactive for TH (25%) or ChAT (7%). Of the synapses formed by ChAT-labeled terminals, 31% (55/176) were with unlabeled perikarya and dendrites; whereas 7% were with perikarya and dendrites with ChAT-I. Also, 21% of the ChAT-labeled terminals contacted the same unlabeled perikarya and dendrite as a TH-containing terminal or were in apposition to TH-labeled terminals (25%). The remaining terminals with ChAT-I were either in apposition to unlabeled or ChAT-labeled terminals or lacked associations with any processes. These findings provide cellular substrates for direct synaptic modulation of (1) cholinergic neurons by both catecholamines and acetylcholine and (2) other non-cholinergic neurons by acetylcholine alone or in conjunction with catecholamines. (Supported by grants MH42834 and HL18974.)

447.18

PERINATAL CHOLINE SUPPLEMENTATION AND THE RAT THALAMOLIMBIC SYSTEM: MORPHOLOGICAL ALTERNATIONS IN GRANULE AND PYRAMIDAL CELLS.

Warren H. Meck and Christina L. Williams. Departments of Psychology, Columbia University and Barnard College. New York, New York 10027.

Choline chloride supplementation during embryonic days (ED) 12-17 and later during postnatal days (PD) 16-30 -- but not PD 0-15 -- has been shown to produce long-lasting facilitation of spatial memory processes in the adult rat. We now report that these two time frames represent anatomically and morphologically distinct phases of choline related influence. Sholl sphere analysis of dendritic trees from Golgi stained sections using a 3-D Eutectic Neuron Tracing System revealed that all three perinatal choline treatments produced a large leftward-shift of the function relating dendritic intersections/ring to sphere radius from the cell body for granule cells in the dentate gyrus. In adult rats (8 months) distributions of dendritic intersections/ring initially centered at a sphere radius of 88 ± 11 mμ from the cell body for the controls were shifted to a sphere radius of 38 ± 6 mμ with no accompanying change in the overall number of dendritic intersections for perinatally choline treated rats. In contrast, only the dendritic trees of pyramidal cells in the CA 3 region of the hippocampus for rats in the ED 5-23 group were affected, whereas, the dendritic branching of pyramidal cells in the lateral posterior and lateral dorsal nuclei of the thalamus was shifted leftward for subjects in the PD 0-15 group. These differential patterns of sensitivity exhibited by neurons in the hippocampus and thalamus during early development can be profitably related to the differences in memory facilitation exhibited by the timing of choline supplementation.

HIPPOCAMPUS AND AMYGDALA: NEUROPHYSIOLOGY II

448.1

MU BUT NOT DELTA OPIATE RECEPTOR ACTIVATION REDUCES FEEDFORWARD AND RECURRENT IPSPs IN HIPPOCAMPAL PYRAMIDAL CELLS *IN-VITRO*.

C.R. Lupica, W.R. Proctor and T.V. Dunwiddie, Dept. Pharmacology, Univ. Colorado Hlth. Sci. Ctr., Denver, CO 80262.

Opiate receptor agonists are thought to excite hippocampal pyramidal neurons through inhibition of GABAergic inhibitory interneurons. We have previously shown that both μ and δ selective opiate agonists produce marked increases in population spike amplitude when applied to superfused hippocampal slices. However, no study has directly compared the actions of these agonists on measures of interneuron inhibition. We compared the effects of the μ selective agonist DAGO and the δ selective agonist DPDPE on IPSPs recorded intracellularly in CA1 pyramidal cells using antidromic (recurrent IPSPs) and subthreshold, orthodromic stimulation (feedforward IPSPs). While the μ receptor agonist DAGO (100 nM) resulted in a significant 50 ± 5% (n=10) reduction in feedforward IPSPs and a significant 48 ± 7% (n= 5) decrease in recurrent IPSPs, the δ receptor agonist DPDPE did not reduce either type of IPSP (n=8 and 6, respectively), at a concentration 5x higher than that which markedly increases population spikes (500 nM). Neither DPDPE or DAGO had any effects upon resting membrane potential, input impedance, or afterhyperpolarizations. The possibility that DPDPE was not acting through the reduction of inhibition in the hippocampus was explored by applying DPDPE or DAGO to hippocampal slices following pretreatment with the GABA-A antagonist bicuculline methiodide (BMI; 30 μM). The excitatory effects of both DPDPE (n=8) and DAGO (n=8) on population spike responses were blocked with BMI. These results suggest that although μ and δ receptor activation mediate diminished GABAergic inhibition in the hippocampus, these receptors do so via different mechanisms.

Supported by NIH grant DA 02702 and the Veterans Administration Medical Research Service.

448.2

IMAGING OF INTRINSIC OPTICAL SIGNALS IN HIPPOCAMPAL SLICES DURING SYNAPTIC ACTIVATION.

D.Hochman and B.A.MacVicar, Neuroscience Research Group, University of Calgary, Calgary, Alberta T2N4N1.

Neuronal activity has been shown to induce changes in intrinsic optical properties of CNS tissue. We have used digital video microscopy to examine whether there are changes in transmittance of light through hippocampal slices during synaptic activity. Synaptic responses from Schaffer collateral stimulation were recorded simultaneously with the acquisition of digitized video images of the CA1 region. Images acquired during stimulation were subtracted from control images. Repetitive synaptic activity was correlated with a progressive increase of light transmission through the stratum radiatum which recovered to control levels several sec after stimulation was terminated. Blocking synaptic transmission with kynurenate (1.5 mM) or 0 Ca^{++}-EGTA perfusate blocked the optical changes indicating that postsynaptic activation was necessary for the signals. Furosemide (5mM), an anion transport inhibitor, reversibly blocked the synaptically-evoked optical changes. Transient changes in optical properties of the slice may represent cellular swelling and be correlated with volume changes in the extracellular space. Supported by MRC (Canada).

448.3

COMPARTMENTAL MODELLING OF THE SPACE CLAMP PROPERTIES OF CA1 HIPPOCAMPAL PYRAMIDAL CELLS

E.W. Stockley* and H.V. Wheal, Department of Neurophysiology, University of Southampton, Bassett Crescent East, Southampton, SO9 3TU. UK.

The technique of recording synaptic currents utilising whole cell patch is gaining widespread interest. However, the space clamp effectiveness of the method may be limited in highly branched neurones such as pyramidal cells. We have simulated the performance of a somatic voltage clamp in a compartmental model based on a serially reconstructed CA1 pyramidal cell (Wheal,H.V & Stockley,E.W, *Soc.for Neurosci.*15:403, 1989).

Synaptic inputs to the model were simulated using either transient (alpha function) conductances or current pulses. The resulting synaptic and somatic currents as well as the voltage profile in the dendrites were calculated for inputs at different positions in the dendritic tree. The voltage deviation from the clamp voltage, and the time course of the somatic current were used as a measure of clamp effectiveness.

Results demonstrate the poor clamp effectiveness for inputs at more distal locations. We have also compared the results from this model with those obtained using branched and collapsed equivalent cylinder models.

Supported by the Wellcome Trust.

448.4

BICUCULLINE AND PHACLOFEN IONTOPHORESIS BLOCK AMYGDALAR PHYSIOLOGICAL RESPONSES TO BASAL FOREBRAIN STIMULATION IN RATS: AN *IN VIVO* STUDY.

L. E. Mello, A. M. Tan* and D. M. Finch. Department of Neurology and Brain Research Institute, University of California, Los Angeles, CA 90024

Amygdaloid responses to electrical stimulation of the basal forebrain were recorded *in vivo* from chloral hydrate anesthetized adult male Sprague-Dawley rats. Extracellular recordings were performed using 5-25 MΩ micropipets filled with 1 M NaCl saturated with Fast Green, attached to a multibarreled micropipet for iontophoresis.

Intracellular recordings showed that responses usually consisted of antidromic activation followed by an EPSP, action potential, and IPSP. Inversion of evoked IPSPs using KCl pipets indicated that the IPSPs were at least partly mediated by chloride influx. Iontophoretic application of bicuculline could induce burst firing that was restricted to the first part of the evoked IPSP, whereas effective application of phaclofen blocked only the last portion of the IPSP. These results were observed for cells in every tested amygdaloid nucleus. Histology showed stimulating electrodes to be in the diagonal band, ventral pallidum, olfactory tubercle, bed nucleus of stria terminalis and nucleus accumbens. In conclusion, the results suggest that inhibition produced by basal forebrain action on the amygdala is at least partly mediated by GABAergic mechanisms. Supported by NIH Grant NS 23074 and FAPESP (Brazil).

448.5

A DIGITAL NEURONAL SPIKE DETECTION AND CLASSIFICATION SYSTEM USING ACTIVITY AND MOBILITY WAVEFORM DESCRIPTORS. **J.D. Bronzino, R.J. Austin-LaFrance and P.J. Morgane.** Dept. of Engineering and Computer Science, Trinity College, Hartford, CT 06106.

We have developed a neuronal spike detection and separation system utilizing the Hjorth waveform descriptors **activity** (power) and **mobility** (slope spread) (**A&M**) to classify neuronal activity derived from multi-unit recordings. The system represents a significant advance in both the speed and accuracy with which spike-like events can be separated and classified by a PC-based, real-time system. The system was tested against the reduced feature matching technique of peak-to-peak amplitude and duration in order to illustrate the versatility of these measures. Multi-unit recordings from hippocampal region CA1 and the dentate granule cell layer were used to determine the ability of **A&M** measures to effectively separate two distinct cell groups recorded during the behavioral states of REM and slow-wave sleep. Classification of spikes was achieved by using feature histograms and cluster plots generated from the **A&M** measures. The results of these comparisons indicate that **A&M** measures can be calculated at a significantly faster speed and provide better characterization of spikes than conventional approaches to real-time spike classification. **(Supported by NIH Grant # R15NS24135-01A1)**

448.6

ENHANCEMENT OF DENTATE GYRUS FIELD POTENTIALS THROUGH GLUTAMATE-INDUCED ACTIVATION OF THE SUPRAMAMMILLARY NUCLEUS (SUM) IN RATS. G.P. Carre and C.W. Harley Dept. of Psychology, Memorial University, St. John's, NF, Canada A1B 3X9

A large number of cells centered in the lateral aspect of the SUM innervate the supragranular layer of the dentate gyrus and send diffuse fibers to Ammon's Horn of the hippocampus. Recently, it has been demonstrated that electrical prestimulation of the SUM enhances perforant path-dentate gyrus evoked field potentials (Mizumori et.al. J. Neurophysiol. 61:15-31, 1989)

Considering the large number of fibers that pass through this region, we investigated the effects glutamatergic stimulation of this region had on dentate gyrus field potentials in urethane-anaesthetized female Sprague Dawley rats. The perforant path was stimulated by a bipolar electrode at a rate of 0.1 Hz (8-25 V), evoking an EPSP and a population spike in the dentate gyrus granule cell layer. L-glutamate (500 mM, typically 100-150nl) was delivered by either pressure injection through a glass micropipette or through a 30 gauge cannula, directed at the SUM. Dependent variables were EPSP slope, latency to the start and peak of the spike, and three measures of spike size: height from first positive potential to the spike peak, height from the spike peak to a tangent, and area under the tangent. Mean values for six events were compared to 95% confidence intervals based on the control period of ten means (10 min.).

Glutamate injection in the area of the SUM associated with hippocampal afferents consistently produced an enhancement of the population spike reflected in all 3 measures of spike size. The spike height (spike start to spike peak), averaged over 1 min., was significantly enhanced from 123 to 161% of the control mean. The period of the enhancement ranged from 2 to above 30 min. No consistent effects were found on EPSP slope or the 2 latency measures. Sites just outside of the hippocampal projection area did not produce spike facilitation.

448.7

OSCILLATORY PROPERTIES OF THE HIPPOCAMPAL FORMATION FOLLOWING REVERSIBLE BLOCKADE OF THE MEDIAL SEPTUM.

L.V. Colom, *S. Nassif-Caudarella, B.H. Bland. University of Calgary, Department of Psychology, Behavioral Neuroscience Research Group, Calgary, Alberta T2N 1N4

The integrity of the medial septal (MS)/vertical limb of the diagonal band of Broca (vDBB) region is critical for the appearance of theta (θ) field activity in the hippocampal formation of the intact animal. θ field activity can be generated in the hippocampal formation by systemic, intraseptal and intrahippocampal infusions of the cholinergic agonist, carbachol. Previous work from our laboratory demonstrated that a "θ-like" field potential can be generated in hippocampal slices perfused with carbachol. We were therefore interested in determining if microinfusions of carbachol in the hippocampal formation of the intact urethane-anesthetized rat could produce θ field activity during a reversible procaine HCl blockade of the MS/vDBB. MS/vDBB blockade was confirmed by electrical stimulation of the dorsomedial-posterior hypothalamic nuclei. Experiments revealed that intrahippocampal infusions of carbachol failed to produce θ field activity when the MS/vDBB was blocked in this manner. However, when microinfusion of carbachol was followed by a microinfusion of bicuculline (a GABA-A antagonist) in the same hippocampal site, a "θ-like" oscillation similar to that seen in hippocampal slices was observed. The oscillations were 3-6 mV with frequencies ranging between 5-12 Hz and had a depth profile similar to that of θ, with a phase reversal in stratum radiatum. The extent to which these oscillations share the same mechanisms as θ field activity, is currently under investigation.

448.8

THE EFFECTS OF INTRASEPTAL INFUSIONS OF PROCAINE ON HIPPOCAMPAL THETA-ON AND -OFF CELLS IN THE URETHANE-ANESTHETIZED RAT. J.W. Smythe, V. H.Lawson*, B. H. Bland. Beh. Neuro Res. Grp, Dept. Psych, Univ. Calgary, Calgary, AB, T2N 1N4

The integrity of the medial septum/vertical limb of the diagonal band nuclei (MS/vDB) is essential for the generation of hippocampal theta (θ) rhythm. Previous research from this laboratory has indicated that θ–on cells are activated by acetylcholine, and we have speculated that θ-off cells are inhibited by GABA. In order to assess these possibilities, we examined the responses of θ-on and -off cells to reversible suppression of the MS/vDB induced by procaine HCl (PRO).

Rats were anesthetized with urethane, and implanted with hippocampal reference and hypothalamic stimulating electrodes, as well as cannulae in their MS/vDBs. Once a cell was isolated and baseline recordings obtained, PRO was injected until 1.0 mA stimulation no longer elicted θ activity.

17 θ-on cells were recorded; 16 phasic and 1 tonic. Their pre-PRO, mean discharge rate during θ was 11.872 ∓ 3.131 Hz, while during large amplitude, irregular activity (LIA) it was 4.465 ∓ 2.756 Hz. At 1 min. post-PRO the discharge rate was 0.345 ∓ 0.431 Hz. By the conclusion of the experiments, the mean rate had risen to 10.128 3.352 Hz during θ, and 4.192 ∓ 2.632 Hz during LIA. 2 θ-off cells were recorded. Their pre-PRO, mean discharge rate during θ was 1.70 ∓2.43 Hz, and during LIA it was 6.284 ∓ 1.953 Hz. At 1 min. post-PRO their mean discharge rate was 5.352 ∓ 2.397Hz.

These data suggest that suppression of the MS/vDB removes excitatory inputs from θ-on cells (presumably cholinergic in nature), and removes inhibitory inputs from θ-off cells (presumably GABAergic). Research is now underway to examine the role of GABA in hippocampal θ field and cell activities.

448.9

SIMULTANEOUS MULTISITE MONITORING OF NEURAL ACTIVITY BY LASER SCANNING MICROSCOPY. P. Saggau+, R. Hiendl* and F. Rucker*. Dept. Physiol. Univ. Munich, FRG. +Div. Neuroscience, Baylor Coll. Med., Houston, TX 77030.

Optical recording with voltage-sensitive dyes has become a powerful tool for investigating neural preparations in which use of multi-electrode approaches would be difficult or impossible. All multisite recordings employing optical methods that have been published so far, made use of non-scanning microscopy (see: Ann Rev Physiol, 51, 1989; Ann Rev Neurosci 11, 1985), due to the multiple technical difficulties of scanning microscopy outweighing its well known advantages.

The new scanning microscope system we have developed is based on a laser, an acousto-optical deflection system, a specially designed inverted-type epifluorescence microscope as well as computer-controlled scanning and detection electronics. The prototype version of this system is capable of recording from up to 100 sites at a rate of 2,000 frames per second, thus enabling the monitoring of fast events such as synaptic and action potentials.

For a first step this system has been employed for multisite monitoring of neural activity in hippocampal brain slice preparations (300-500μm). After placing the slices in a recording chamber, a bipolar electrode for stimulation was positioned in the Schaffer collaterals while an extracellular microelectrode was used for recording from the CA1 region (Saggau et al., Neurosci Lett 69, 1986). After staining the preparation with a voltage-sensitive dye (25μM RH-414 for 30min) the recording system was set up by positioning the optical recording sites under visual control. When using a 40x objective lens the spot size is 10μm and the maximally scanned area 150μm x 150μm. Due to the fast scanning rate and the achieved signal-to-noise ratio simultaneous monitoring of neural activity of many sites was possible.

The extension of the described system to confocal microscopy with its improved spatial resolution of all three dimensions is under development.

(Supported by a grant of the Heidenhain Foundation to P. Saggau)

448.10

A MODEL OF THE ENHANCED LOCATION-SPECIFIC FIRING OF CA1 PYRAMIDS DURING TRANSLATIONAL MOVEMENTS. S. E. Fox. Dept of Physiol, SUNY Health Sci Ctr, Brooklyn, NY 11203.

CA1 pyramidal cells in rats that are engaged in a simple foraging task show location-specific firing. That is, they fire in a small sector of the environment, the "place field", whereas they are nearly silent in other regions. This location-specific firing is enhanced during translational movements that are associated with the presence of the atropine-resistant component of the hippocampal theta rhythm. In unrestrained rats, this enhancement of location-specific firing appears as both an *increase* in firing rate within the place field and a *decrease* in firing rate outside the field. A model of CA1 synaptic mechanisms that is capable of such a discrimination is described below.

It is assumed here that spatial information arrives in CA1 via the direct projection from entorhinal cortex to the distal apical dendrites of the pyramids. During movement, afferents from the basal forebrain cause increased firing of interneurons that produce inhibition at the somata of the pyramids (Stewart & Fox, TINS 13: 163, 1990). This is supported by current source density (CSD) analysis of theta rhythm (Brankačk & Fox, Neurosci Abstr 13: 1331, 1987), and by earlier extracellular recordings from interneurons. The CSD also shows a large sustained sink in the distal apical dendrites during movement-related theta rhythm, suggesting *excitation* of pyramids at this site. The tonic distal dendritic excitation in combination with tonic somatic inhibition can explain the enhanced location-specific firing. Spatial information arriving at the distal dendrites, in the presence of tonic excitation, activates the dendritic spike trigger zone, causing the cell to fire in spite of the somatic inhibition. The somatic inhibition blocks activation by the more diffusely localized "non-spatial" afferents that are able to fire the cell outside the place field during motionless behaviors. (Supported in part by NIH NS17095.)

448.11

THYROTROPIN-RELEASING HORMONE (TRH) IS RELEASED FROM HIPPOCAMPAL SLICES AFTER ELECTROCONVULSIVE SHOCK (ECS). S.M. KNOBLACH, S. DURBIN, M.J. KUBEK, Depts. of Anatomy, Psychiatry and Program in Medical Neurobiology, Indiana Univ. & VA Medical Centers, Indianapolis, IN 46202.

TRH levels (Ann. N.Y. Acad. Sci. 553:286,'89) and TRH mRNA (this meeting) are elevated in rat hippocampus after ECS. We used an in vitro K^+ stimulation preparation to examine whether increases in tissue TRH coincide with enhanced release. Sham ECS and ECS (3x) rats were killed either 12 or 24 h after the last treatment. Sliced hippocampi were perfused with oxygenated Kreb's solution modified as follows: 1) high K^+ + Ca^{++} (HKC); and 2) high K^+ + EGTA (HKE).

Fractions were collected during a 30 min stimulation with HKE followed by HKC and analyzed for TRH via RIA. No significant release occured in either group during HKE stimulation. TRH was increased in the HKC fraction of ECS animals compared to sham after both 12hrs (1.12+/-0.06 vs 0.66+/-0.05pg/min. $p<0.005$) and 24hrs (2.11+/-0.32 vs 0.94+/-0.16pg/min. $p<0.02$). HKC fractions collected 24hs after ECS contained more TRH than those obtained 12 hrs after ECS ($p<0.025$). This is the first report of TRH release from an extrahypothalamic site. Moreover, these results confirm that elevated TRH seen after ECS represents TRH available for release and thus may have a significant effect on receptor modulation. Supported by NS-25661 & VA Research.

448.12

BRAINSTEM LESIONS THAT SELECTIVELY ELIMINATE THE HIPPOCAMPAL THETA RHYTHM DURING REM SLEEP. R.P. Vertes. Center for Complex Systems, Florida Atlantic University, Boca Raton, FL 33431

In an earlier report (Vertes, J. Neurophysiol. 42: 214, 1979), we identified neurons in the brainstem that fire selectively during the two states in which the theta rhythm is present in the hippocampus of the rat -- waking-movement and REM sleep. Cells of this type were predominantly localized to the pontine reticular formation (PRF). In a follow-up study (Vertes, J.Neurophysiol. 46:1140, 1981), we showed that hippocampal theta could be very effectively elicited with PRF stimulation, and concluded that the source(s) for the generation of hippocampal theta reside within the PRF.

In the present report, we examined the effects of brainstem lesions (electrolytic and kainic acid-induced) on the hippocampal theta rhythm. We found that large bilateral lesions of the PRF encompassing virtually the entire nucleus pontis oralis (RPO) and the rostral pole of pontis caudalis (RPC) completely eliminated theta of waking and REM. Unexpectedly, we also found that relatively small bilateral lesions confined to the ventrolateral quadrant of the PRF eliminated the theta rhythm of REM but not that of waking. The theta of waking remained completely intact. The lesions were located ventrolaterally at the RPO-RPC border, ventral to the motor nucleus of the trigeminal nerve, in the general region of the A5 noradrenergic cell group. No sites were identified at which lesions selectively eliminated the theta of waking.

The results support the position that the PRF is the source for the generation of theta and further suggest there are separate brainstem systems for the elicitation of theta of waking and REM sleep similar to separate septal systems for the production of theta during these two states (Monmaur et al., Physiol. Behav. 23:471, 1979). Supported by NIMH grant MH 45075.

448.13

DEPTH PROFILES OF AN ATROPINE-RESISTANT COMPONENT OF THE HIPPOCAMPAL THETA RHYTHM IN URETHANE ANESTHETIZED RATS. Mark Stewart and Steven E. Fox, Dept. Physiol., SUNY Health Science Center, Brooklyn, NY 11203.

The hippocampal theta rhythm, first characterized by its distinct behavioral correlates, has been separated into atropine-sensitive and atropine-resistant components. The atropine-sensitive component of the urethane-induced theta rhythm is well known. Recently, an atropine-resistant component of this theta rhythm was described (Stewart and Fox, Brain Res. 500: 55, 1989). We constructed depth profiles of the atropine-resistant component of the theta rhythm in urethane anesthetized rats by averaging the hippocampal EEG recorded from different depths, triggered by the rhythmic activity of an atropine-resistant medial septal cell (Stewart and Fox, J. Neurophysiol. 61: 982, 1989). Amplitude profiles showed peaks in the basal and mid-apical dendrites of CA1, and near the granule ceil layer. Amplitude minima were located at the pyramidal cell layer and in the apical dendrites of the dentate granule cells. Phase shifts of 90° occurred at the two amplitude minima so that the signals in the basal dendrites of CA1 and the dentate granule cell layer were phase-reversed. The largest amplitude peak occurred between the two 90° phase shifts suggesting that two dipoles are not sufficient to account for the profiles. GABAergic septohippocampal afferents probably generate the amplitude peaks near the pyramidal and granule cell layers, but the origin of the peak in stratum radiatum is unknown.
(Supported in part by NIH grants NS17095 and NS07117.)

NEUROETHOLOGY: AVIAN SONG

449.1

EXPERIENCE-DEPENDENT CHANGES OF SEXUAL PREFERENCES AND SPINE DENSITY IN A FOREBRAIN AREA OF THE ZEBRA FINCH. H.-J. Bischof and A. Rollenhagen, Dept. of Ethology, Univ. of Bielefeld, PO-box 8640, 4800 Bielefeld 1., F.R.G.

If a male zebra finch after 60 days of isolation courts a female the first time in its life, the birds under certain circumstances change their initial sexual preference. In this study we prove this change of preference and show that by such exposure to a female substantial changes of neuron morphology occur in one of four areas of the forebrain, which have been shown previously to be activated during this first courtship encounter.

100 day old Bengalese finch reared zebra finch males which were after 60 days of isolation first exposed to a zebra finch female for 7 days followed by 7 days of exposure to bengalese finches, changed their preference to zebra finches, whereas males receiving the reverse exposure stabilized their initial Bengalese finch preference.

The first 7 day exposure to a female after 100 days of isolation resulted in an increase in spine density in one subpopulation of ANC-neurons compared to controls.

We suspect that the features which are learned as releasers for courtship behaviour in the course of the sensitive period have to be verified and consolidated in the first courtship encounter. The increase in spine density of ANC may be a reflection of this consolidation process, although it may also be a consequence of social experience per se. Supp. by Deutsche Forschungsgemeinsch.

449.2

MIDBRAIN AND TELENCEPHALIC CONTRIBUTIONS TO VOCAL CONTROL IN ZEBRA FINCHES D.S.Vicario and H.B.Simpson. The Rockefeller University, New York, NY 10021

Electrical stimulation has been reported to elicit vocalizations consistently from the avian midbrain, but rarely from the telencephalon. In songbirds, the vocal pathway includes telencephalic nuclei HVC and RA, the midbrain intercollicular complex (ICo), and the hypoglossal subregion (nXIIts) projecting to the syrinx, or vocal organ. We have found that long microstimulation trains (1-4s) elicit vocalizations from HVC, RA, and ICo. Within ICo, the dorso-medial nucleus (DM) has the lowest threshold (<10μA). DM stimulation elicits loud, repeated vocalizations at short latency that resemble calls. In the caudal telencephalon, similar low thresholds for stimulation are obtained from HVC and RA. Vocalizations are softer, with longer latencies. They are acoustically complex, and can resemble syllables from the bird's own song in duration and/or acoustic features. Vocalizations elicited from RA lose their complex features if the syringeal nerve is cut. Since RA projects to both DM and nXIIts, the remaining vocalizations may depend on the RA to DM projection. These results suggest that some characteristic features of song are determined in the path from HVC to the syrinx, and can be evoked by a simple stimulus.

We have also used stimulation to target lesions of DM in order to explore its contribution to normal vocalization in male and female zebra finches. We have previously shown that lesions of either HVC or RA abolish song and strip the male long call of its learned features, but do not affect the female long call. In contrast, bilateral lesions of DM in males do not abolish song but primarily affect its temporal features: the motif duration is longer and sequence anomalies may increase, but only minor acoustic changes are seen. The long call is also slightly affected. In females with DM lesions, long call duration is dramatically affected. This suggests that vocal production in females is primarily due to the midbrain pathway, whereas in males, midbrain and telencephalic contributions interact.

449.3

MASCULINIZATION OF ZEBRA FINCH VOCAL NUCLEI DEPENDS ON TIMING OF HORMONE TREATMENT H.B. Simpson and D.S. Vicario. The Rockefeller University, NY, NY 10021.

Telencephalic nuclei HVC and its two targets, RA and X, make critical contributions to the learned vocalizations produced by male zebra finches. Females do not produce these learned vocalizations; HVC, RA, and X are smaller in volume; HVC and RA are not connected. We have shown that early estrogen (E) treatment alone is sufficient to masculinize the vocal behavior of females. We now present data showing that the timing of E treatment critically determines the spectrum of neuroanatomical masculinization.

Seven females received E pellets at birth. All produced male-like vocalizations. In 5 (3@100μg, 2@50μg), the volumes of RA and X were dramatically larger than in normal females, confirming earlier reports. HVC projected to both RA and X as shown by the anterograde tracer PHA-L. The other 2 (100μg) received testosterone (T) as adults and, in both, the frequency of singing increased; in 1, T treatment stabilized the temporal structure. However, T had no further effect on the volume of RA or X.

Six other females were treated with E at birth, but the pellets were removed at 15 days. In 4 of these, RA was small, but HVC projected to RA in only 2. In contrast, X was large in volume and was innervated by HVC. None of these females produced male-like vocalizations. The 2 remaining females received T as adults and then produced song-like vocalizations of poor quality. RA was small but innervated by HVC. X was large and innervated.

These data suggest 1) that the innervation and volume of X can be masculinized independently from that of RA, and 2) that RA innervation and RA size are not always associated. We are now exploring the possible role of endogenous T in both behavioral and anatomical masculinization by treating females with Flutamide as well as E. (MH-40900; GMO-7739)

449.4

ACQUISITION OF A CONSPECIFIC SONG DISCRIMINATION IS FACILITATED BY TESTOSTERONE IMPLANTS IN CASTRATED ZEBRA FINCHES. J. Cynx & F. Notttebohm. Rockefeller Univ. Field Research Center, Millbrook, NY 12545.

Adult male zebra finches learned to discriminate between conspecific songs in fewer trials when trained in the summer than in the winter. We speculated that this difference resulted in part from differences in testosterone (T) levels. To test this hypothesis, castrated zebra finches were implanted with either T-filled or empty silastics. They were then trained to discriminate between two canary song segments. Hopping to the food dispenser after hearing one song segment was rewarded with access to seeds; hopping to the dispenser after hearing the other song segment resulted in a time out. All birds required approximately the same number of trials to acquire the discrimination. Each T-implanted bird was then paired with an empty-silastic implanted bird. Each pair was then trained--singly and using the same procedure--to discriminate between their own two songs. T-implanted birds acquired the discrimination in fewer trial than their paired controls. The results suggest that testosterone facilitated acquiring a conspecific, but not hetereospecific song discrimination.

449.5

ROLE OF LMAN IN POST-CRITICAL PERIOD SONG LEARNING IN ZEBRA FINCHES. **R.G. Morrison & F. Nottebohm. Rockefeller Univ., NY, NY 10021.**

Male zebra finches learn their songs between post-hatching days 30 and 80, but this "critical period" for song learning can be extended by isolating juveniles from singing adults (Eales 1985, 1987). Removal of the lateral magnocellular nucleus of the anterior neostriatum (LMAN) of normally reared zebra finches disrupts song development, but does not affect the songs of adult males (Bottjer et al., 1984). To determine whether the effects of LMAN removal depend on age or on completeness of learning, we evaluated the role of LMAN in isolates after the normal end of the critical period.

Male zebra finches were visually isolated from conspecifics from day 28 to day 86, when their songs were recorded. In group I an adult conspecific male tutor was then added to each cage; in group II each isolate was given bilateral LMAN lesions and then an adult male was added. All birds were kept with their tutors for 4-5 months, and songs recorded periodically.

After 4-5 months of tutoring, intact isolates had learned an average of 4 elements of their tutors' songs; in contrast, lesioned isolates retained their songs but showed no evidence of new learning. These results suggest that in adult zebra finches that can still modify their songs, LMAN is necessary for song learning but not for maintenance of song already developed.

449.6

REORGANIZATION OF CRYSTALLIZED SONG AND ITS CENTRAL CONTROL IN THE ADULT ZEBRA FINCH. H. Williams, J.R. McKibben*, and M.A. Esposito*. Biology Dept., Williams College, Williamstown, MA 01267.

Adult male zebra finches *(Taeniopygia guttata)* are "critical period" learners, and crystallize their songs at 90 days. Adult song remains fixed and is not altered by deafening (Price, P., *J. Comp. Physiol. Psychol.*, 93:260, 1979). Injuring either of the tracheosyringeal (ts) nerves (which control the vocal organ) induces a deficit in the phonology of syllables forming adult male song; a greater deficit is seen after right than after left nerve injury (Crane, L.A., Price, P., & Nottebohm, F., unpubl. ms.).

After unilateral ts nerve injury, long-term changes in the temporal patterning of adult males' songs were seen. Syllables were deleted, remaining portions of the song were elided, and new syllables were added. Syllables with call-like phonology were less likely to be deleted from and more likely to be added to the song. Deletions were most often composed of contiguous chunks of syllables. Changes in the temporal patterning of song occurred during specific periods following nerve injury, were completed within 100 days after nerve injury, and were not dependent upon or affected by nerve regeneration. The resulting new song patterns were stable, remaining unchanged up to one year later. Neither side nor severity of injury had a substantial effect upon the type and number of changes that were induced within the song.

Subsequent lesioning of the central song system nucleus HVC indicates that the reorganization of song circuitry responsible for the changes in crystallized song occurs in a different manner in adult and developing male zebra finches. Young males are able to transfer all song control functions to the left hemisphere, but adult males do not make this transfer and so must presumably use crossing pathways in the descending song system; such pathways are not thought to be important for production of normal song (Paton, J.A. & Manogue, K.R., *J. Comp. Neurol.*, 212:329, 1982).

These results indicate that a form of vocal plasticity remains even after song learning is completed, though this plasticity may be restricted to a subset of song characteristics. The limitations on the types of possible changes in adult song may reflect 1) how syllables are centrally represented and 2) the potential for neural plasticity in the central song system of "critical period" learners.

449.7

EFFECTS OF EARLY AUDITORY DEPRIVATION ON THE DEVELOPMENT OF AVIAN VOCAL CONTROL NUCLEI. M. J. Burek, K.W. Nordeen and E.J. Nordeen. Dept. Psych., U. Rochester, Rochester, NY 14627

Altering sensory experience can change the timing of neural events associated with "sensitive" developmental periods. In male zebra finches, the sensitive period for vocal learning correlates with large changes in the size and number of song-related neurons. Between 20 and 65 days of age, new neurons are added to the hyperstriatum ventralis pars caudalis (HVc) and Area X, and the robust nucleus of the archistriatum (RA) increases in volume. In contrast, the lateral magnocellular nucleus of the anterior neostriatum (lMAN) loses nearly 50% of its neurons during this same period. To determine how auditory experience during the sensitive period affects these changes, male zebra finches were deafened or sham operated at 10 days of age and sacrificed at either 25 or 65 days. Nuclear volume and neuron number were measured for HVc, RA, lMAN and Area X.

Early deafening did not alter the development of neuron number in HVc, Area X or RA, but did attenuate neuron loss from lMAN. At 65 days, nuclear volume and neuron number in HVc, Area X, and RA did not differ between intact and deafened birds. In contrast, while both groups lost lMAN neurons between day 25 and 65, at the latter age lMAN neuron number was significantly less in intact than in deafened birds. These data suggest that auditory experience during the sensitive period regulates neuron death in lMAN. To confirm these findings and identify the neurons spared by auditory deprivation, we are extending this study using retrograde and anterograde tracing techniques.

449.8

DEVELOPMENTAL CHANGES IN A THALAMIC NUCLEUS INVOLVED WITH VOCAL LEARNING IN ZEBRA FINCHES. F. Johnson & S.W. Bottjer. Dept. Biol., USC, Los Angeles, CA 90089

Nucleus lMAN in the zebra finch forebrain plays a critical role during the period of song learning in juvenile males. lMAN undergoes a decline in overall nuclear volume and neuronal number between 20 and 35 days of age. In contrast, other forebrain nuclei involved in song control (e.g., HVc, Area X, RA) actually increase in volume and/or neuronal number during vocal development. Neither projecting to an efferent target nor the ability to concentrate androgenic hormones appear to enhance survival of lMAN neurons.

We have been investigating the possibility that afferent projections to lMAN are involved in lMAN neuronal attrition. Based on previous work showing that lMAN receives a single afferent input from nucleus DLM of the thalamus, we sought to answer two questions: (1) At what time during development do DLM fibers innervate lMAN? (2) Does DLM undergo morphological changes during development that parallel those of lMAN? To address the first question, crystals of DiI were placed into DLM bilaterally in formalin-fixed brains taken from male zebra finches of various ages (13-50 days old). Results indicated that DLM fibers arrive in lMAN early in development, as anterograde label was identified over lMAN in birds as young as 13 days old. To address the second question, DLM volume was measured in 20, 25, 30, 35, and 40 day-old male zebra finches. While DLM volume did not differ among 20, 25, and 30 day-olds, it was significantly smaller in 35 and 40 day-olds. Thus, thalamic afferent innervation of lMAN precedes, and volumetric decline in DLM follows, the onset of neuronal attrition in lMAN.

449.9

BLOCKING STEROID HORMONES DURING VOCAL LEARNING EXTENDS SUSCEPTIBILITY TO DEAFENING IN ZEBRA FINCHES. S.W. Bottjer & E. Foster. Dept. Biol., USC, Los Angeles, CA 90089

Deafening disrupts song behavior in juvenile male zebra finches, indicating that they require auditory feedback of vocalizations in order to develop normal song. However, deafening exerts little or no disruptive effect in adult birds (>90 days) that are producing stereotyped song. We have previously reported that blocking steroid hormones prevents normal development of learned vocal behavior, and that delayed exposure to testosterone beyond the normal period for vocal learning permits delayed development of normal song production. This pattern of results raises the possibility that low levels of steroid hormones extend the period during which zebra finches can learn to produce specific song patterns, and that high levels of testosterone and/or the development of stereotyped motor patterns of song act to close the period for learning.

To test this idea, males were castrated at 20 days of age and received continuous exposure to an anti-androgen (flutamide) and/or an anti-estrogen (tamoxifen) until c. 185 days of age. Birds were then deafened via removal of the cochleae, and song behavior was recorded pre-operatively and for a period of one to two months following surgery. In all cases deafening produced significant disruption of the song patterns even though birds were well beyond the normal period of susceptibility to deafening. Thus, preventing development of stable song behavior by blocking access to sex steroids seems to extend the period of reliance on auditory feedback.

449.10

LESIONING AXONAL CONNECTIONS OF A THALAMIC NUCLEUS DISRUPTS SONG DEVELOPMENT IN ZEBRA FINCHES. K.A. Halsema & S.W. Bottjer. Dept. Biol., USC, Los Angeles, CA 90089

Previous investigations suggest that discrete portions of the neural song system in male zebra finches play a critical role in the development of song, but not in the production of the stable adult song pattern. Lesions of either lMAN or Area X in juvenile birds severely disrupt song production, whereas the same type of lesion in adult animals producing a fully stereotyped vocal pattern has little or no effect. Area X projects to lMAN trans-synaptically, via the thalamic nucleus DLM. In order to investigate the role of DLM in song learning, we made electrolytic lesions of a forebrain region containing both the afferents to DLM from Area X, as well as the efferents from DLM to lMAN in young males between 40 and 70 days of age. In all instances when these two sets of fibers were damaged, vocal production was significantly disrupted. Surprisingly, unlike lesions of lMAN, the disruptive effect of this type of lesion on the final adult song did not fall off as the bird developed a more stereotyped song pattern. Analysis of the adult song in birds over 100 days of age revealed that lesions made as late as 70 days produced vocal deficits comparable to lesions made earlier in development. In contrast, disruption of these fibers in adult birds left song production unaltered. The X-DLM-lMAN circuit appears to be developmentally regulated as lesions made only early in ontogeny disrupt song production. However, lesions of the afferents to lMAN are effective later in vocal development than are lesions of lMAN itself. Thus, not all portions of this circuit "orchestrate" song development within the same time frame.

449.11

NEURONAL RESPONSES TO SONGS AND ARTIFICIAL STIMULI IN OVOIDALIS AND HVc: A CONNECTIONIST MODELLING APPROACH. S. C. Bankes* and D. Margoliash. Rand Corp., Santa Monica, CA and Dept. Anatomy, Univ. of Chicago, Chicago, IL.

A quantitative relationship between central neuronal responses to simple artificial and complex natural stimuli has rarely been achieved. We conducted recordings in urethane-anesthetized zebra finches and backpropagation modelling experiments to investigate this issue.

Auditory neurons in the song system nucleus HVc responded well to song, systematically preferring the bird's own song. Some but not all HVc neurons also responded to various artificial stimuli tested. In contrast, ovoidalis neurons responded to tones as well as song, and exhibited other 'classical' properties.

For modelling, modified backprop architectures we are experimenting with utilize trainable feature detectors with limited temporal windows whose response across the duration of the stimulus is integrated by output units whose temporal response properties are also constrained. A two phase training technique where initial weights for feature detectors are set via pre-training using short duration artificial stimuli has thus far been the most effective. The current implementations consistently show excitability to the stimuli which evoke responses in the neurons, but have some susceptibility to false alarms. These limitations may be resolved with use of different, higher order, or fully recurrent architectures.

These results can provide a quantitative framework for identifying non-linear neuronal properties, transformations in the auditory pathway, and can provide a rationale when using a limited stimulus repertoire.

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449.12

PERCEPTION OF BIRDSONG BY FEMALE ZEBRA FINCHES & CANARIES. S.J. Clark and F. Nottebohm. The Rockefeller University, Field Research Center, Millbrook, NY 12545.

Birdsong serves two roles - the spacing of males and the wooing of females. If female mate choice is influenced by song, then female preferences will shape the predispositions of males to learn some songs over others. However, males and females need not perceive song in the same way. Here we report our first attempt at defining the song parameters perceived and preferred by female zebra finches and canaries.

Using playbacks of song to estradiol-implanted female canaries and zebra finches, we were able to elicit copulation solicitation displays in the laboratory and used this assay to determine what features a song must possess in order to be perceived by the females as functionally adequate. Female zebra finches gave displays in response to conspecific song but not heterospecific song. The songs of conspecific males raised in isolation were much less effective than the songs of normally raised males in evoking solicitation displays. Using a computer to manipulate and modify songs revealed that female zebra finches appear to attend to a hierarchy of cues with proper syllable morphology being more important than correct sequence of syllables. Female canaries also discriminate between conspecific and heterospecific songs. Female canary song does elicit displays but is not as effective as male song. We are now searching for features of conspecific song that can be heightened so as to elicit super-normal responses in females. It is our hope that finding such features will tell us a good deal about the mechanisms involved.

DRUGS OF ABUSE: CANNABINOIDS, NICOTINE AND PCP

450.1

EFFECTS OF DELTA-^{9}-TETRAHYDROCANNABINOL ON VENTRAL TEGMENTAL A_{10} DOPAMINE NEURONS IN THE RAT. S.Levenson* and E.D. French, Dept. Pharmacol, Univ. Arizona, Coll. Med. Tucson, AZ 85724.

Marijuana is the second most widely abused drug in the United States. Yet there is little known about its actions on the A_{10} mesocorticolimbic dopamine systems which are considered of fundamental importance for the positive reinforcing qualities of many drugs of abuse. Thus in these studies, we set out to determine the effects of Δ^9-THC, the major psychoactive constituent of marijuana, on A_{10} neuronal activity.

Under chloral hydrate anesthesia extracellular recordings were made from single A_{10} neurons during i.v. injections of Δ^9-THC, cannabidiol (a nonpsychoactive cannabinoid) or vehicle. Evaluation of the cumulative dose-response curves showed that Δ^9-THC caused a dose-dependent increase in firing rate reaching a maximum 19% change at 4 mg/kg. However, larger doses led to some attenuation of the increase, possibly due to the depressant effects of the vehicle. Identical doses of cannabidiol produced virtually no effect (max.= +3%), while equivalent volumes of vehicle (4 ml/kg) resulted in a progressive decrease in rate to -13%. Ongoing experiments indicate that Δ^9-THC's stimulatory effects are naloxone (10 mg/kg) insensitive. Also, apomorphine-induced inhibitions of A_{10} firing do not appear to be different following vehicle or Δ^9-THC treatment. Since the vehicle in which Δ^9-THC is solubilized may have a counter-opposing action, Δ^9-THC may actually be a more potent activator of mesocorticolimbic dopamine neurons than demonstrated in this preparation.

Thus Δ^9-THC can significantly change A_{10} activity, like other drugs of abuse. It remains to be determined whether this action might underlie some of marijuana's abuse liability and psychotropic properties.

450.2

FURTHER EVIDENCE FOR Δ^9-TETRAHYDROCANNABINOL AS A DOPAMINE REUPTAKE BLOCKER: BRAIN MICRODIALYSIS STUDIES. E.L. Gardner, W. Paredes*, and J. Chen. Depts of Neuroscience and Psychiatry, Albert Einstein Col. of Med., New York, NY 10461.

We have previously shown that Δ^9-tetrahydrocannabinol (Δ^9-THC), the psychoactive ingredient of marijuana, augments brain-stimulation reward (Gardner et al., *Psychopharmacology* 96:142, 1988) and enhances presynaptic dopamine (DA) efflux in caudate-putamen (Ng Cheong Ton et al., *Brain Res.* 451:59, 1988) and in nucleus accumbens and medial prefrontal cortex (Chen et al., *Soc. Neurosci. Abstr.* 15:1096, 1989). Further, the time-dynamics of Δ^9-THC's action on DA efflux suggested an action analogous to that of a DA reuptake blocker (Ng Cheong Ton et al., *Brain Res.* 451:59, 1988). The present experiments were undertaken to further explore that possibility, using intracerebral microdialysis in awake freely-moving rats. We now report that haloperidol (0.1 mg/kg,i.p.) pretreatment (1 hr prior to Δ^9-THC) has a synergistic effect on Δ^9-THC's enhancement of presynaptic DA efflux in nucleus accumbens. Δ^9-THC (1.0 mg/kg, i.p.) pretreatment 1 hr before haloperidol also had a synergistic effect on presynaptic DA efflux. Tetrodotoxin perfused locally at 10^{-5} M abolished the synergism between haloperidol and Δ^9-THC. Since impulse-induced facilitation of DA release underlies the synergistic effect between DA receptor blockers and DA-reuptake inhibitors (Westerink et al., *Eur. J. Pharmacol.* 135:123, 1987), the present data add further evidence that Δ^9-THC may act as a DA reuptake blocker in brain reward circuitry.

450.3

DELTA-9-TETRAHYDROCANNABINOL DOES NOT AFFECT EFFLUX OF MESOTELENCEPHALIC DOPAMINE AS MEASURED BY IN VIVO MICRODIALYSIS IN FREELY MOVING RATS. S.D. Oddie, E. Castañeda, D.E. Moss and I.Q. Whishaw. Departments of Psychology, University of Lethbridge, Lethbridge, Alberta T1K 3M4, Canada, and University of Texas, El Paso, Texas 79968 U.S.A.

We used in vivo microdialysis to evaluate whether delta-9-tetrahydrocannabinol (THC) alone increases the efflux of dopamine (DA) from the striatum or nucleus accumbens and whether THC enhances the increase of DA produced by either amphetamine or fluphenazine. Dialysis samples were collected during: 1) baseline, 2) after 1 mg/kg or 10 mg/kg THC (or vehicle), and 3) after 1.5 mg/kg d-amphetamine SO_4. Locomoter responses and turning behavior were measured during these three conditions. In an additional experiment 10 mg/kg THC (or vehicle) plus 0.3 mg/kg fluphenazine 2HCl was administered immediately after resting-state (baseline) measures. THC produced no change in extracellular concentrations of DA, DOPAC, and HVA, or in 5-HIAA, and had no effect on DA release produced by amphetamine and fluphenazine. There were no behavioral differences between groups during any treatments. Thus, increasing activity at mesotelencephalic DA synapses is an unlikely basis for the propensity to self-administer marijuana by humans.

450.4

QUANTITATIVE TOPOGRAPHIC EEG STUDIES OF CHRONIC THC ABUSE. F.A. Struve, J.J. Straumanis*, G. Patrick* and Y. Raz*. Neurophysiology Lab, LSU Sch. of Med., Shreveport, LA 71130-3932

In a pilot study (Clin. EEG.,20:6-23,1989) 10 chronic daily THC users with no THC access and negative urines were contrasted with 10 patient non-users and 10 normal non-users using multiple quantitative EEG measures. THC users had significant elevations of Absolute and Relative Power and Coherence of alpha over frontal cortex ("HYPERFRONTALITY OF ALPHA") as well as other quantitative EEG signs. These findings have been successfully replicated with new samples of 17 THC users, 21 patient non-users and 12 normal non-users. Also using all 80 Ss and all quantitative EEG variables as potential predictors, a discriminant function analysis yielded a 95% correct THC user Vs non-user classification. A JACK-KNIFE replication yielded 93.8% correct classification. In expanded studies Discriminant Scores correlated positively with Duration of THC use in years and Exposure (Duration x Daily Amount) but negatively with duration of Abstinence in years. Finally, the spectral profile (Absolute Power in 1 Hz intervals from 1 to 25 Hz) was found to be shifted to slower frequencies for THC users as compared to non-users

450.5

DELTA-9-TETRAHYDROCANNABINOL EFFECTS ON TONE EVOKED POTENTIALS RECORDED FROM RAT NEOCORTEX. Sam A. Deadwyler, Eric M. Blalock*, Jian Mu* and Robert E. Hampson, Department of Physiology and Pharmacology, Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, North Carolina 27103.

Previous studies from this laboratory have shown that administration of delta-9-tetrahydrocannabinol (THC) impairs behavioral detection of a single tone in rats performing a signal detection task (Heyser *et al.*, SN Abstr., 14:1030, 1988). The same THC doses (0.5-2.0 mg/kg) did not alter neural activity associated with auditory system detection of the tone (Campbell *et al.* JPET, 239:941-945, 1986) but reduced hippocampal neural activity associated with behavioral responding to the tone. We recorded cortical EPs from skull electrodes in the rat to determine whether the effect of THC on processing of behaviorally-relevant sensory information affects transmission of cortical information to the hippocampus.

Cortical EPs evoked by brief (4 msec) tone "pips" revealed several short-latency auditory system-related potentials (<10 msec). Large negativities at 12 msec (N12), 25 msec (N25), and 40 msec (N40) were also evoked by the tone. Following 2.0 mg/kg THC injection, the short-latency auditory system potentials and N25 were unchanged, the N12 was slightly decreased, and the N40 was reduced in amplitude and increased in latency.

In the behaving rat, cortical EPs evoked by tone stimuli showed the N40 deflection and a later positive (P140) deflection (Hampson *et al.*, SN Abstr., 15:81, 1989). In the signal detection tasks, detection of the tone signal was accompanied by a large N40 and P140, and nondetection of the tone was associated with a decreased N40 and P140. Since the principal effect of THC on behavior was decreased tone detection, and N40 was decreased in nondetect trials, the decreased N40 amplitude following THC administration may indicate that THC blocks processing of sensory information between primary auditory receiving areas and neocortical association areas which project sensory information to the hippocampus. The effects of chronic THC exposure (4-8 months) on EPs in the behaving rat will be examined. *[Supported by grants DA00119, DA03502 & DA04441 to S.A.D.]*

450.6

DELTA-9-TETRAHYDROCANNABINOL INHIBITION OF THE NEUROENDOCRINE RESPONSE OF ADULT MALE RATS TO SEXUALLY RECEPTIVE FEMALE CONSPECIFICS. I. Gher*, L.L. Murphy, R.W. Steger and A. Bartke*. Department of Physiology, Southern Illinois University, School of Medicine, Carbondale, IL 62901.

The effects of delta-9-tetrahydrocannabinol (THC), the major psychoactive component of marijuana, on the rapid increases in luteinizing hormone (LH) and prolactin (PRL) levels and hypothalamic norepinephrine (NE) turnover in adult male rats exposed to sexually receptive female conspecifics were investigated. Adult male Sprague-Dawley rats were given p.o. either sesame oil vehicle (controls) or THC (5.0 mg/kg b.w.) and 40 min post-treatment were either placed in an empty cage or a cage containing a sexually receptive female. Males were sacrificed 20 min after exposure (60 min after vehicle or THC treatment) for blood and tissue collection. Some rats received α-methylparatyrosine 30 min prior to sacrifice for NE turnover determinations. In rats given sesame oil vehicle and exposed to a receptive female, there was a significant increase in LH (non-exposed, 0.373 ± 0.055 ng/ml vs exposed, 0.623 ± 0.082 ng/ml; $p < 0.05$) and PRL levels (non-exposed, 9.4 ± 1.5 ng/ml vs exposed, 61.4 ± 14.5 ng/ml; $p< 0.01$) and in median eminence NE turnover rates. In THC-treated rats, basal LH levels (0.199 ± 0.032 ng/ml) and NE turnover were significantly reduced when compared to controls ($p < 0.05$). Moreover, rats treated with THC and exposed to female conspecifics failed to show the rapid increase in LH and PRL levels or in NE turnover rates. In conclusion, these findings indicate that THC inhibits the increase in LH and PRL normally measured in male rats exposed to sexually receptive females and further suggest that THC mediates these actions by altering hypothalamic catecholaminergic activity. (Supported by DA 03875 and DA 05042).

450.7

NALOXONE REVERSES BUT DOES NOT PREVENT DELTA-9-TETRAHYDROCANNABINOL-INDUCED INHIBITION OF PULSATILE LH SECRETION IN OVARIECTOMIZED RATS. P. Keller*, M. Kohli* and L.L. Murphy. Department of Physiology, Southern Illinois University, School of Medicine, Carbondale IL 62901.

Although the ability of delta-9-tetrahydrocannabinol (THC), the chief constituent of marijuana, to suppress LH secretion in laboratory animals has been clearly documented, the mechanism of this THC action has yet to be elucidated. In the present study, the ability of naloxone (NAL), an opiate receptor blocker, to prevent or reverse the effects of THC on pulsatile LH secretion was examined in ovariectomized Sprague-Dawley rats. Approximately 4 weeks post-ovariectomy, blood samples were drawn, via indwelling intra-atrial cannulae, every 10 min for 60 min pre- and 120 min post-vehicle (control) or THC (0.5 mg/kg b.w., iv) treatment at time 0 for LH determinations. Rats were administered NAL (2 mg/kg b.w., iv) or saline at 20 min pre-THC or vehicle treatment, at time 0, and /or at 20 min post-treatment. The results indicated that THC treatment alone significantly reduced plasma LH levels by 20 min post-treatment to 50-75% of control values ($p<0.05$) through 90 min. Pre-treatment with NAL did not prevent THC-induced suppression of LH release. However, NAL administration at 20 min post-THC treatment reversed the inhibitory effect of THC on LH levels 40-90 min post-THC. Moreover, when NAL was administered prior to THC, concomitant with THC administration, and 20 min post-THC treatment, both the magnitude of LH suppression by THC and the duration of suppression were significantly attenuated. These data suggest that endogenous opiate peptides play a role in the ability of THC to inhibit pulsatile LH secretion, however, the possibility that other CNS mechanisms (ie, norepinephrine) are involved is strongly implicated. (Supported by DA 05042)

450.8

EFFECTS OF Δ^9-TETRAHYDROCANNABINOL ON REGIONAL CEREBRAL BLOOD FLOW IN THE RAT. A.S.Bloom, S.A. Fuller*, E.A.Stein. Departments of Pharmacology and Psychiatry, Medical College of Wisconsin, Milwaukee, WI 53226.

Δ^9-Tetrahydrocannabinol (THC), the principal psychoactive ingredient in the marijuana plant, produces a wide range of behavioral and physiological effects. Cannabinoid binding sites have been found to be widely but heterogenously distributed throughout the brain. In order to better determine functional sites of action for the cannabinoids, we have now examined the effects of THC on regional cerebral blood flow (rCBF) in the rat. Conscious rats were injected IV with either 4 or 16 mg/kg of THC or its Emulphor-ethanol vehicle 30 min prior to sacrifice. [^{14}C]Iodoantipyrene was used to determine rCBF according to the method of Sakurada et al. Blood flow was altered in 49/53 regions measured. Two patterns of change were observed. A significant dose related decrease in rCBF was seen in the hippocampus (up to 30%) and its major input and output areas such as the lateral septum, entorhinal cortex and the dentate. In contrast, a dose-related biphasic increase (4 mg/kg>16 mg/kg) in rCBF was seen in such areas as the anterior, lateral and ventromedial hypothalamus and other limbic structures involved in autonomic regulation. Other areas such as the medial septum and claustrum were unaffected. It is significant that the areas where rCBF is altered are involved in the major behavioral and physiological effects of THC such as impairment of short-term memory and increased autonomic activity. The results of this study indicate that alterations in rCBF may be a sensitive technique for the localization of cannabinoid sites of action within the brain. (Supported in part by NIDA grants DA03725 and DA05012)

450.9

TIMEOUT FROM AVOIDANCE: A NOVEL TEST OF DRUG EFFECTS ON NEGATIVELY-REINFORCED BEHAVIOR. M. Galizio, E. Gregg*, L. Kelly*, R. L. Shriner*, and R. D. Shytle*. Dept. of Psychology, UNC-Wilmington, Wilmington, NC 28403.

Theoretical analysis of the neuropharmacological basis of negative reinforcement has been hampered by difficulties in generating baseline performances comparable to those used with positive reinforcement. The timeout from avoidance procedure permits the study of negatively-reinforced behavior in rats under schedules ordinarily used only with positive reinforcement. Rats were trained on concurrent schedules where responses on one lever postponed shock (avoidance) and responses on another lever produced signaled periods of timeout from avoidance during which the avoidance schedule was suspended. Timeout was programmed on a variable-interval 45-s or a variable-ratio 15 schedule, and generated patterns of behavior similar to those produced by comparable schedules of positive reinforcement. Morphine depressed responding on the timeout lever at doses that increased or did not affect avoidance. Amphetamine selectively increased responding on the timeout lever, but chlorpromazine and buspirone non-selectively decreased responding on both levers. The finding that the effects of some drugs (morphine and amphetamine) depended on the type of negative reinforcer maintaining behavior has implications for theories regarding the neurochemical substrates of negative reinforcement.

450.10

CHANGING ENVIRONMENTAL CUES UNIQUELY ASSOCIATED WITH DRUG DELIVERY REDUCES TOLERANCE TO NICOTINE-INDUCED ANOREXIA AND CORTICOSTERONE RELEASE. A.R. Caggiula, L.H. Epstein*, S.M. Antelman* S.Saylor* and K.A.Perkins*. Depts. of Psychology and Psychiatry, Univ. of Pittsburgh, Pittsburgh, PA 15260

We have previously shown that changing environmental cues associated with drug delivery disrupts tolerance to the analgesic and anorectic effects of nicotine in rats. Here we asked whether the effectiveness of nicotine in activating the hypothalamic-pituitary-adrenocortical system, as measured by blood corticosterone levels, can also be brought under environmental control. Secondly, we were interested in further characterizing the nature of this environmental control. Male rats developed tolerance to the anorectic and corticosterone elevating effects of daily injections of 0.33 mg/kg of nicotine bitartrate. When cues uniquely associated with drug delivery were then changed, both behavioral and corticosterone tolerance were disrupted. When there were no unique cues associated with drug delivery per se, changing the environment had no effect on tolerance to either of nicotine's actions. Thus conditioning principles apply to tolerance of the neuroendocrine, as well as behavioral effects of nicotine.

450.11

SYSTEMIC NICOTINE INDUCES NEURONAL FOS IMMUNOSTAINING IN DISCRETE BRAIN REGIONS. S.M Sagar. Dept. of Neurology, Univ. of California, and VA Medical Center, San Francisco, CA 94143.

Nicotine, 2 mg/kg s.c, was administered to Long Evans rats, which were sacrificed 1 hr later and processed for Fos immunocytochemistry using a polyclonal antibody that recognizes authentic Fos and Fos-related antigens. Brain regions with high levels of Fos immunostaining following nicotine administration include: the hypothalamic paraventricular and supraoptic nu. (magnocellular neurons), medial habenula, superior colliculus, interpeduncular nu., nu. solitarius, locus coeruleus, and scattered cells in the granule cell layer of the cerebellum. Other regions, including the thalamus, that have high levels of nicotine binding, fail to display Fos immunostaining. These observations demonstrate that (1) nicotine is capable of stimulating Fos synthesis in vivo, although the mechanism is not necessarily direct; (2) some, but not all, neurons with nicotinic receptors synthesize Fos in response to nicotine; and (3) Fos immunocytochemistry may be a useful tool to investigate the effects of nicotine on the central nervous system.

450.12

PHENCYCLIDINE-INDUCED ALTERATIONS OF EXTRAPYRAMIDAL AND LIMBIC NEUROPEPTIDE Y SYSTEMS. L.P. Midgley, L.G. Bush, J.W. Gibb and G.R. Hanson. Dept of Pharmacology and Toxicology, University of Utah, Salt Lake City, UT 84112

Phencyclidine-HCl (PCP) causes many effects including behavioral changes which resemble the negative and positive symptoms of schizophrenia. PCP is known to interact with a number of transmitter systems and has recently been identified as an NMDA antagonist. We previously reported that administration of PCP significantly reduces striatal neuropeptide Y (NPY) levels in rats: some of these NPY changes also occurred after treatment with the non-competitive NMDA antagonist, MK-801. In the present study, we observed that PCP administration also caused significant declines in NPY levels in the nucleus accumbens and frontal cortex, but not in the substantia nigra. In order to elucidate the nature of these responses, selective D-1 (SCH 23390), D-2 (sulpiride), sigma (rimcazole), 5-HT-3 (ICS205-930) antagonists and a drug which depletes serotonin (PCA) were administered alone and in combination with PCP. Only SCH 23390 attenuated the PCP-induced change in NPY levels in all sructures examined. These results suggest that D-1, but not D-2, 5HT or sigma receptors play a role in PCP-induced changes of extrapyramidal and limbic NPY systems. (Supported by grants DA 00869 and DA 04222).

450.13

DETECTION OF BEHAVIORALLY ACTIVE DOSES OF PHENCYCLIDINE IN RAT BRAIN AND CSF BY HPLC-EC. C.J. Drebing, G.A. Gerhardt, W.F. Phillips*, S. Brock*, and R. Freedman. VA Medical Center, Denver, CO 80220; Dept. of Psychiatry, Univ. of Colorado Health Sciences Center, Denver, CO 80262.

Phencyclidine (PCP) is a drug of abuse that has many behavioral effects. Members of our group have previously shown PCP to increase dopaminergic neurotransmission in the prefrontal cortex of rats (Gratton et al., 1987), but little is known of the behaviorally relevant concentrations of the drug.

We injected male Sprague-Dawley rats, anesthestized with chloral hydrate, with PCP concentrations of 1-5 mg/kg. At one hour the CSF was collected and the brain removed. PCP was analyzed in the samples after a simple acid, then basic buffer-hexane extraction for brain and a basic buffer-hexane extraction for CSF. Dried reconstituted samples were injected into an HPLC-EC with pH 5.5 acetate acetonitrile mobile phase on a cyano 3μ column. Detection was at +.80v on an ESA 5100A Coulochem with a 5010 detector. Each sample required 10 min. elution time.

CSF and cortex PCP concentrations were dose dependent. PCP concentrations in the cortex ranged from 0.4-2.7 μM and PCP concentrations in the CSF ranged from .07-.30μM. The ratio of the PCP concentrations in CSF versus cortex was .15. Supported by NIDA grant, DA-02429.

451.1

COPULATING ACTIVITY MODIFICATIONS IN MICE PRENATALLY TREATED WITH DIAZEPAM. Márquez-Orozco, M.C. Hernández-Alvarez, L.A.I., Márquez-Orozco, A., Moralí de la Brena, G. Dept. of Embriology. School of Medicine, UNAM; Biomedical Research Unit, CMN, IMSS. 04510 México, D.F., MEXICO.

Prenatally diazepam treated mice fetuses, young and adult mice show alterations in CNS structure and in behavior. We evaluated the effect of these alterations upon the copulating activity of CD-1 strain mice prenatally treated with diazepam. One group of female mice was i.p. treated with diazepam (2.7 mg/kg/d) from the 6th to the 17th of gestation and another group received an equivalent volume of saline sol. Their offsprings were wet-nursed. On the 6th month, the spontaneous male sexual activity to receptive females was tested in 3 sessions (1/week). Tests were performed during the dark stage of the photoperiod and videorecorder under red light. Precopulating and copulating activities were evaluated. Duration of the introduction of the mount series with ejaculation (MSWE) tended to be longer in the treated mice, incidence of interruptions of intravaginal penetration and the number of interruptions of penile penetrations into MSWE were also greater. Treated animals showed a significant larger incidence of falls and pauses during mount series with intromission ($p < 0.05$). Results indicate alterations in their capacity to mantain an erection or a motor activity failure that interferes with this response.

451.2

CONDITIONED RESPONSE TO BARBITURATE INJECTION WITH THE OPERANT PARADIGM.
P.M. Duncan, T.Buck* and E. Muggenthaler*. Psychology Department, Old Dominion University, Norfolk, VA 23508.

Conditioned responses (CR) to barbiturate treatment were investigated in rats lever pressing for food pellets by presenting an auditory-visual stimulus (CS) 3 min before IP injection of na pentobarbital 15 mg/kg (exp group,n=9) or water (ctrl group,n=8). The exp rats completely stopped responding during the 20-min post-injection period. During 12 conditioning days, the exp group developed CRs to the preinjection CS as indicated by response suppression to 28% of baseline rate, significantly ($p<.05$) different from the 80% ctrl group mean. Sessions in which water only was injected in both groups revealed significant CRs (lower exp group resp rates) to the injection procedure. Both the pre and post-injection CRs lowered response rate, as did the actual drug treatment. Visual observation suggested that the CS elicited locomoter activity which interfered with lever pressing, implying that a compensatory CR was elicited by stimuli associated with the barbiturate treatment.

451.3

CARBAMAZEPINE DOES NOT AFFECT ALPRAZOLAM WITHDRAWAL.
W.R. Galpern, D.J. Greenblatt*, R.I. Shader*, L.G. Miller. Div. of Clinical Pharmacology, Depts. of Psychiatry and Pharmacology, Tufts-New England Medical Ctr., Boston, MA 02111.

Discontinuation of the triazolobenzodiazepine alprazolam (ALP) can produce a clinical "withdrawal" syndrome. An analogous syndrome of behavioral hyperactivity and $GABA_A$ receptor upregulation has been reported in mice (Lopez et al., Neuropharmacol, 29:237, 1990). Some clinical evidence indicates that carbamazepine (CBP) may be useful in limiting the symptoms associated with alprazolam discontinuation. To evaluate the effects of CBP on ALP discontinuation in a mouse model, we assessed the effects of an anticonvulsant dose of CBP (50 mg/kg/d) administered after ALP discontinuation, on open-field activity, benzodiazepine binding in vivo and in vitro, TBPS binding, and GABA-dependent chloride uptake in cortex. ALP (2 mg/kg/d) or vehicle were administered for 1 week by implanted osmotic pump; 24 hrs after pump removal, a second pump containing CBP or vehicle was implanted for 1 week. CBP administered after 1 wk treatment with vehicle did not alter open-field activity, benzodiazepine or TBPS binding, or GABA-dependent chloride uptake. As previously reported, ALP followed by vehicle was associated with increases in motor activity, benzodiazepine binding, and chloride uptake 2 and 4 days after ALP discontinuation. Similar findings were observed in mice treated with CBP after alprazolam. Thus, CBP at an anticonvulsant dose does not affect behavioral or neurochemical effects of alprazolam discontinuation.

451.4

EFFECTS OF EARLY EXPERIENCE ON DRUG CHOICE IN A NOVEL ANIMAL MODEL OF MULTIPLE SUBSTANCE ABUSE.
B.Zimmerberg and M.B.Brett*. Psychology Dept., Williams College, Williamstown, MA 01267.

Individuals vary in rates of initiation and maintenance of drug self-administration behavior. One source of these individual differences may lie in differential early experience. Long-Evans rats were weaned at 25 days of age into one of three environmental conditions: social isolate (SI), sibling double-housed (DH), or enriched environment (EE). At 85 days of age, subjects were singly housed and given a choice between drinking a stimulant (d-amphetamine, 0.025 mg/ml), a depressant (barbital, 1 mg/ml) or water for two hours daily. Drug choice was affected by both housing and sex. SI males consumed significantly less depressant than any other group. When forced to choose between the two drugs without a water alternative, EE and SI males showed a clear preference for the stimulant, and SI and DH females for the depressant. These results suggest that males and females respond differently to the stress of social isolation, and appear to contradict the "tension-reduction" hypothesis used to explain depressant drug intake, at least for males.

451.5

TOLERANCE TO PHENOBARBITAL AND CROSS-TOLERANCE TO MUSCIMOL GIVEN IVT TO RATS.
J.A. Richter, S.L. Gatto* & B. Glick*, Depts. Pharmacology and Psychiatry, Indiana Univ. School of Medicine, Indianapolis, IN 46202.

We have found previously that many phenobarbital (PheB) effects including the loss of righting reflex (LRR) and hypothermia can also be elicited by ivt administration of muscimol. In order to test the hypothesis that PheB induces these effects by a GABAergic mechanism we have asked if cross-tolerance occurs between these two drugs. Studies were done in male Wistar rats with indwelling guide canulae directed to the lateral ventricle. These methods were previously used by Mycek and Brezenoff (1976) who found tolerance to the LRR induced by PheB after 4 days of 4x/day ivt injections. We found that 1250 ug NaPheB 4x/day for 4 days induced tolerance to the LRR but not to the hypothermia. Similar chronic administration of 1600 ug NaPheB induced tolerance to the hypothermia and LRR. When these rats were tested on the 5th day with 1 ug muscimol, the hypothermic effect of muscimol was reduced significantly, but the LRR induced by muscimol was not greatly altered. These results support a GABAergic mechanism for barbiturate hypothermia. Further experiments will test whether longer periods of chronic PheB treatment will induce cross-tolerance to the other effects of muscimol, and if chronic muscimol induces cross-tolerance to PheB effects. (Supported by PHS R01 DA00796).

451.6

BEHAVIORAL TOLERANCE TO MULTIPLE CHRONIC DOSES OF MIDAZOLAM ON FIXED RATIO RESPONDING.
M.Kallman, S.Bowen*, E.Ventress*, R.Anderson*, M.Durnam* and S.Fowler. Depts. of Psychol. & Pharmacol., U. of Miss., University, MS 38677.

This investigation explored the importance of chronic dose on behavioral tolerance and behavioral withdrawal with midazolam(MZ). Forty-eight male rats were trained to respond on a fixed ratio (FR) 30 schedule of reinforcement during daily 20 min. sessions. An initial dose effect curve was determined (0.3-10.0 mg/kg) and redetermined following 30 days on chronic exposure. During the chronic exposure period half the rats (N=24)were exposed to drug 15 min. presession(PRE) and half were exposed 30 min. postsession (POST) with 6 rats from each PRE and POST group exposed to 0,1.32, 3.0 or 10 mg/kg during the chronic period. FR performance was observed for 60 days following termination of drug exposure. Behavioral tolerance did develop to MZ with the degree of behavioral tolerance dependent on MZ dose. Behavioral withdrawal was also determined by time of chronic drug exposure and MZ dose delivered.These findings support the contention that behavioral factors are important in the development of tolerance and withdrawal and that behavioral factors are optimal when chronic drug delivery does not produce complete debilitation. Support DA05253.

451.7

WITHDRAWAL AND CROSS TOLERANCE IN RATS MADE DEPENDENT ON CHLORDIAZEPOXIDE. S. L. Pugh*, S. Abdel-Malek*, and M. W. Emmett-Oglesby, Department of Pharmacology, TCOM, Fort Worth, Texas, 76107-2690

Rats were trained to detect the anxiogenic drug pentylenetetrazole (PTZ; 20 mg/kg) using a two-lever food-reward choice task. When tested with chlordiazepoxide (20 mg/kg; CDP) in combination with the benzodiazepine antagonist, flumazenil (40 mg/kg), they selected the saline lever. Subsequently, they were treated with CDP (20 mg/kg/8-hr) for 7 days, and then they were retested with the CDP/flumazenil combination at various times after stopping chronic administration of CDP. In contrast to the results obtained prior to chronic CDP, this combination of drugs now resulted in predominately PTZ-lever selection at 8 and 24 hr after the last chronic dose of CDP. Tests at 48 hr or later again resulted in saline-lever selection. A second group of animals was trained to discriminate PTZ (20 mg/kg) from midazolam (1.0 mg/kg) from saline. Using a cumulative dosing procedure, in acute dose-effect tests, PTZ substituted for PTZ, and midazolam, CDP and diazepam substituted for midazolam in a dose-related manner; in addition, low doses of drug resulted exclusively in either responding on the saline lever or responding on the lever that was appropriate for that drug (e.g., PTZ-lever following PTZ). These subjects were then given CDP (20 mg/kg/8-hr) for 7 days and subsequently tested for their detection of midazolam. The dose-effect curve shifted approximately two-fold to the right at 8 hr after the last chronic injection of CDP. Thus, these results support the hypothesis that chronic CDP produces dependence and tolerance that can be detected in an animal model of subjective drug effects.

Supported by grant DA RO1 3521.

451.8

DISCRIMINATIVE STIMULUS PROPERTIES OF FLUMAZENIL DURING CHRONIC CHLORDIAZEPOXIDE TREATMENT. G.A. Rowan and M.W. Emmett-Oglesby, Department of Pharmacology, TCOM, Ft.Worth, TX 76107-2690

Rats were trained using operant methods to discriminate the stimulus properties of flumazenil (2.5 mg/kg) while maintained on a liquid diet containing chlordiazepoxide (CDP) (100 mg/kg/daily). The animals were given CDP in divided doses: they were trained 6 hours after a 25 mg/kg dose, and after training they were given the remaining 75 mg/kg of CDP. In dose-effect testing flumazenil substituted for the training stimulus in a dose-dependent manner (0.64 - 2.5 mg/kg), producing an ED50 of 0.94 mg/kg. The animals were then taken off chronic CDP. When tested with vehicle the 8th day after withdrawal from CDP the animals responded with a 67% flumazenil-lever selection. In contrast, when tested 14 days after withdrawal the response to vehicle was not significantly different than pre-withdrawal vehicle responding (17% drug lever selection). At this point, the training dose of flumazenil was retested and the animals failed to produce flumazenil-lever selection, indicating that the effects of flumazenil alone were different in the non-dependent subject. These data also indicate a partial substitution between 8-day post-chronic CDP (spontaneous withdrawal) and the flumazenil stimulus trained against the background of CDP (precipitated withdrawal). The animals were then placed back on chronic chlordiazepoxide and trained for an additional three months. Subsequently, the dose effect curve for flumazenil (0.16 - 2.5 mg/kg) was redetermined; the curve was not significantly different from the initial dose-effect curve (ED50 of approximately 0.64 mg/kg), demonstrating a stable discriminative stimulus produced by flumazenil. These data are consistent with having trained the discriminative stimulus properties of precipitated withdrawal from dependence on a benzodiazepine. This research was supported by DA05367 and DA03521.

451.9

EVIDENCE THAT THE BENZODIAZEPINE RECEPTOR PARTIAL AGONIST Ro 16-6028 HAS MINIMAL ABUSE AND PHYSICAL DEPENDENCE LIABILITY J.R. Martin[1], A. Kuwahara[2*], I. Horii[2*], J.-L. Moreau[1*], F. Jenck[1*], J. Sepinwall[3] & W. Haefely[1]. [1]Pharmaceut. Res. Dept., Basel, [2]Tox. & Path. Dept., Kamakura and [3]Pharmacol. & Chemother. Dept., Hoffmann-La Roche Inc., Nutley, N.J.

To assess dependence liability, squirrel monkeys received Ro 16-6028 (RO), alprazolam (A) or vehicle po divided into 3 equal doses at 8 h intervals each day over 11 days. Benzodiazepine receptor (BZR) antagonist challenge (.25 mg/kg Ro 15-3505 iv) was given 5 h, 24 h, and 48 h after the 31st and final administration and observations made for 2 h. Pronounced convulsions were observed in 2/4 and 4/4 of the monkeys receiving 1 or 3 mg/kg A daily, respectively. In contrast, only 0/4, 1/4, and 2/4 of the monkeys receiving 3, 10, or 30 mg/kg of RO daily exhibited any pronounced convulsions, respectively. Given the much greater potency of RO than A in diverse pharmacological tests and the relatively low incidence of severe precipitated withdrawal symptoms after the former, RO clearly exhibits lower dependence potential than the BZR full agonist A. This pattern of results was repeated in DBA/2J mice receiving 2, 6, or 20 mg/kg A, 20,60, or 200 mg/kg RO or vehicle divided into two equal daily oral doses for 17 days and challenged with Ro 15-3505 given 5 h after the final administration. Convulsions were precipitated in 60-75% of mice in groups treated with the two higher doses of A, whereas none were induced in any of the RO groups.

To assess its reinforcing effect, RO was offered to 4 cynomolgus monkeys which had previously been trained to self-administer pentobarbital iv on a FR10 schedule during a daily 6-h session. In repeated substitution tests (consisting of at least 5 sessions), RO was shown to lack reinforcing effects at all doses tested (0.001-0.06 mg/kg per injection). Furthermore, these doses were also inactive in 5 monkeys tested in a self-administration initiation paradigm. (For comparison, it has been found that oral RO disinhibited punished operant responding in squirrel monkeys with a MED ≤ 0.02 mg/kg). Thus, RO was not found to be self-administered within a behaviorally active dose range.

451.10

DISCRIMINATION OF THE STIMULUS PROPERTIES OF THE BENZODIAZEPINE INVERSE AGONIST METHYL-6,7-DIMETHOXY-4-ETHYL-BETA-CARBOLINE-3-CARBOXYLATE (DMCM). L.G.Kirby, G.A.Rowan and I.Lucki. Depts. of Psychiatry and Pharmacology, University of Pennsylvania, Philadelphia, PA 19104.

Rats (N=6) were trained to discriminate the stimulus properties of the benzodiazepine (BZ) receptor inverse agonist DMCM from saline in a conditioned taste aversion paradigm. On a drug trial, water-deprived rats were injected with DMCM (.6 mg/kg IP), allowed access to a .25% saccharin solution for 30 min, and then injected with LiCl (1.8 mEq/kg IP). On nondrug trials, saline injections bracketed the drinking period. Unconditioned controls (N=6) were treated with DMCM but never received LiCl. Acquisition of the discrimination was detected after as few as 5 pairings of DMCM with LiCl. In addition, DMCM produced a dose-dependent reversal of the preference for saccharin over water in a two-bottle choice test after DMCM-LiCl pairings, but not in controls.

The inverse agonists beta-CCE (10-18 mg/kg) and FG-7142 (3.2-18 mg/kg) fully substituted for the effect of DMCM, as measured by the reversal of saccharin preference. Partial substitution for DMCM was shown by the BZ receptor antagonist CGS 8216 (3.2-10 mg/kg) and the non-BZ convulsant pentylenetetrazol (11-20 mg/kg). The BZ agonists chlordiazepoxide (.32-5.0 mg/kg), diazepam (.32-10 mg/kg), and alprazolam (0.1-3.2 mg/kg) all failed to generalize significantly to the DMCM stimulus.

451.11

SELECTIVE BREEDING FOR NEGATIVE CONTRAST; PRELIMINARY DATA. C.F. FLAHERTY, K.L. Krauss*, G.A. Rowan, M. Weaver* & P.S. Grigson.
Psychology Dept., Rutgers Univ., New Brunswick, NJ 08903

We selectively bred rats differing in degree of negative contrast effect when shifted from 32% to 4% sucrose. Shift ratio (lick frequency on first postshift day/lick frequency on last preshift day) was used as the selection criterion. Reliable differences between the two lines (large contrast (LC) and small contrast (SC)) developed over six filial generations. Selection in the direction of contrast larger than the general population was much more pronounced than selection towards small contrast -- an outcome which reflects the distribution of individual differences in a large sample of unselected rats. The two lines also differ in sensitivity to the contrast-alleviating effects of midazolam -- with the drug reducing contrast in the LC rats but having no effect in the SC rats. The two lines also differ in open field activity (LC rats ambulate and rear more than SC rats), but they do not differ in defecation frequency.

The two lines may provide a vehicle for analyzing behavioral and neurochemical correlates of sensitivity to absolute and relative reward effects. The lines may also differ in sensitivity to anxiolytic drugs and, possibly, other drugs which interact with reward systems.

451.12

EFFECT OF ACUTE PENTOBARBITAL ON EXCITATORY AMINO ACID EVOKED-ELEVATIONS OF CYCLIC GUANOSINE 3',5' MONOPHOSPHOSPHATE IN CULTURED CEREBELLAR GRANULE CELLS. W.W. Morgan, J.L. Bermudez* and S.M. Davis*. Dept. Cellular and Structural Biology, Univ. of Texas Hlth. Sci. Ctr. at San Antonio, TX 78284-7762.

Depressant barbiturates suppress the stimulatory effects of the excitatory amino acids on neuronal activity and perhaps act preferentially on activations mediated via kainate- as opposed to NMDA-related receptors (Collins, G.G.S., Neuropharmacology, 26:167, 1987). To investigate this latter effect further, granule cells were cultured from 8 day old rats. Cultures (9 DIV) were preincubated in buffer or in buffer containing Na+ pentobarbital for 10 min before the addition of kainate or NMDA (12.5-100μM). One minute later each reaction was terminated, and cyclic GMP was determined by RIA. Pentobarbital (200μM) markedly suppressed the elevations of cyclic GMP induced by 25-75μM kainate to near the control value while the effect of 100μM kainate was only partially suppressed. The elevation of cyclic GMP produced by 25μM kainate was significantly reduced by dosages of pentobarbital as low as 10μM, but lower dosages remain to be tested. Similar effects of pentobarbital were observed on NMDA-induced elevations of cyclic GMP. These results demonstrate the suppressive effects of pentobarbital on excitatory amino acid-mediated neuronal stimulation, but, as yet, suggest no selectivity for receptor subtype. Supported by NIDA # 00755.

451.13

CHRONIC BENZODIAZEPINE (BZ) TREATMENT REDUCES GABAERGIC INHIBITION IN HIPPOCAMPUS. X-H. Xie and E.I. Tietz. Dept. of Pharmacology, Medical College of Ohio, Toledo, OH 43699

GABA/BZ complex function was examined 48 hr after 1 week chronic flurazepam (FZP) treatment by testing GABA-mediated inhibition in in vitro hippocampal slices. Male rats (200-280 gm) were offered FZP in the drinking water (100 mg/kg X 3 dy;150 mg/kg X 4 dy). Field potentials elicited by Schaffer collateral stimulation (.1 msec monophasic pulse) were recorded (2M NaCl filled glass pipette, 2-5 mΩ) from CA1 pyramidale cells. Stimuli resulting in ½ maximal evoked responses were determined in treated and control (10.8±.6 V vs. 10.8±.5 V) slices for paired-pulse stimulation (inter-pulse interval (IPI), 10-200 ms). Test responses (P2) were measured as a fraction of conditioning responses (P1). Threshold for a 1 mV response (control, 7.9±.4; treated, 7.7±.4 V) and P1 amplitude (3.1±.3 vs 2.8±.1 mV) did not differ (p>.05). All 13 slices from 6 treated rats showed significantly (p<.01) reduced inhibition at all IPI between 10 ms (-67%) and 40 ms (+3%) in comparison to 12 control slices (n=6; -91% and -44%). The significant shift in IPI_{50} in treated (17.0 ms) vs control (33.5 ms) slices suggests reduced local inhibition at a time after chronic BZ treatment when residual drug level is negligible and BZ anticonvulsant tolerance is still apparent. The findings suggest modulation of GABA/BZ complex function underlies BZ tolerance and shows that the hippocampal slice is useful for studying BZ tolerance mechanisms. Supported by grants DA04075 and DA02194.

EPILEPSY: KINDLING I

452.1

PHARMACOLOGICAL PROFILE OF SUBMAXIMAL CORNEAL ELECTROSHOCK KINDLING: LACK OF INVOLVEMENT OF NMDA RECEPTORS? EW Harris, GC Garske, EF Cregan, LR Freedman and GC Palmer, Fisons Pharm., Div. R & D, Box 1710, Rochester, NY 14603.

Rats receiving repeated submaximal corneal electrical stimulation (60Hz, 8mA x 2sec) exhibit a progression of seizures reminiscent of amygdala kindling (Kupferberg, 1989, Epilepsia 30, S1, S51). The time course and pharmacology of "corneal kindling" have been examined.

Rats were stimulated 1 & 2 hr post oral dosing for 5 days, followed by a 2 day wash out and retest. Vehicle-treated rats reached Class IV-V seizures after 5 days. Established seizures were blocked by phenobarbital (30 mg/kg), carbamazepine (100 mg/kg) or the NMDA antagonist MK-801 (0.33 mg/kg). Seizure progression during kindling was slowed by MK-801, phenobarbital, valproate (708 mg/kg) or clonazepam (1 mg/kg). However, seizure severity jumped markedly with the first test after wash-out of MK-801 or phenobarbital, approximating that in vehicle-treated rats; wash-out of clonazepam or valproate revealed only a slight increase in severity. Kindling was not slowed by phenytoin, remacemide or FPL 13950 (40, 60 & 46 mg/kg, respectively); the latter two block NMDA-induced seizures in mice (ED_{50}=57 & 30 mg/kg). Established seizures also were not blocked by phenytoin, remacemide or FPL 13950 (200 mg/kg).

Corneal kindling differs pharmacologically from MES or PTZ seizures, and some drugs thought to retard kindling (including NMDA antagonists) may only mask its expression.

452.2

KINDLING INCREASES THE APV AND CNQX SENSITIVE COMPONENTS OF SYNAPTIC RESPONSES IN BASOLATERAL AMYGDALA (BLA). **D.G. Rainnie, E.K. Asprodini, & P. Shinnick-Gallagher,** Dept. of Pharmacology, Univ. of Texas Medical Branch, Galveston, TX 77550.

Kindling induces burst firing responses in the amygdala. The purpose of these experiments was to analyse the subthreshold synaptic responses underlying this bursting behaviour. Stimulation of the stria terminalis (ST) or lateral amygdaloid nucleus (LA) evokes a multiphasic waveform, consisting of an EPSP, a fast IPSP and a slow IPSP recorded at -60mV using intracellular recording techniques. The EPSP consists of a fast (rise-time 10-90%: 6± 1mS) CNQX (10μM) sensitive component which is enhanced with membrane hyperpolarisation. In the presence of CNQX (10μM) a slow (rise-time 19± 1.5mS) APV (50μM) sensitive component is revealed which is reduced with membrane hyperpolarisation.

Following kindling, increases in both glutamatergic components of the EPSP are observed. In 50μM APV, stimulus intensities evoking a CNQX sensitive EPSP (15± 3.5mV) in control (C) neurones cause burst firing in kindled (K) neurones. Similarly, in CNQX (10μM) significant increases in the APV sensitive component (3.0± 0.3mV, K; 1.4± 0.3mV, C; p = 0.012; -70mV) were recorded at lower stimulus intensities (mean: 24V, C; 10V, K). This APV sensitive EPSP is reduced with membrane hyperpolarisation. At all membrane potentials tested, no significant changes in membrane input resistance were observed following kindling. In addition, firing frequency in response to a transient (100ms) depolarising current step (0.4nA) shows no significant difference (4.6± 0.7, C; 4.1± 0.6, K). Furthermore, APV sensitive EPSPs, recorded in CNQX and bicuculline, in control were smaller than those in kindled neurones. These data suggest that changes in voltage dependent Mg^{2+} block, postsynaptic conductance, firing frequency and/or loss of GABAergic shunt conductance could not account for the enhanced glutamatergic EPSPs. However the data could be explained by pre- and/or post-synaptic changes in glutamatergic transmission. Supported by NS 24643.

452.3

THE NMDA-ANTAGONIST, MK-801, REDUCES THE AMOUNT OF GRANULE CELL INHIBITION PRODUCED BY PERFORANT PATH KINDLING. ME Gilbert. NSI Technology Services Corp., RTP, NC, 27709.

Electrical kindling of the perforant path or dentate hilus produces a reduction in inhibition as indexed by paired pulse stimulation. Antagonists of the NMDA-receptor subtype of glutamate delay the development of kindling. The present experiment examined the development of enhanced inhibition in saline- and MK-801-treated rats during kindling of the perforant path. Kindling was induced by daily delivery of stimulus trains (2 s, 60 Hz, 1.0 pulse duration, 800 μ a) to the perforant path 30 min following 0 or 1.0 mg/kg MK-801 (ip). Pairs of stimulus pulses were delivered at 8 interpulse intervals (IPI) ranging from 20 to 1000 ms. The intensity of the conditioning pulse was set at approximately 75% of the asymptotic population spike for a given subject. Over the course of kindling development, this intensity was adjusted to match the size of the population spike at baseline. As previously reported, MK-801 increased AD thresholds and the number of sessions required to reach Stage 5 seizures. Paired pulse functions monitored before kindling (baseline), and 24 hr after the 1st, 2nd, 6th and 15th AD indicated that MK-801 reduced the amount of inhibition that developed during kindling of control subjects. The effects were most pronounced at IPIs ranging from 70-150 ms which result in paired pulse facilitation under baseline conditions. The enhancement of a late period of inhibition occurring between 200 and 1000 ms was also decreased by MK-801. Thus in addition to the suppression of potentiation in excitatory projection pathways that accompanies kindling, NMDA-antagonists may also suppress potentiation of inhibitory circuits.

452.4

NMDA RECEPTOR BLOCKADE PREVENTS LENGTHENING OF AFTER-DISCHARGES AND LOSS OF PAIRED PULSE INHIBITION INDUCED BY RAPIDLY RECURRING HIPPOCAMPAL SEIZURES (RRHS) METHOD OF KINDLING. Jaideep Kapur[1] and Eric W. Lothman[2], Dept. of Neurology, Medical College of Virginia, Richmond, VA 23298 and Depts. of Neurology and Neuroscience, University of Virginia, Charlottesville, VA 22908.

These experiments examine the role of NMDA receptor activation in prolongation of afterdischarges observed in rapidly recurring hippocampal seizures (RRHS) method of kindling. We have previously shown that delivery of RRHS to awake rats causes a rapid kindling and that RRHS in awake and urethane anesthetized rats causes progressive lengthening of afterdischarges and a loss of paired pulse inhibition. Paired pulse inhibition is dominantly GABA mediated.

Pretreatment with NMDA receptor antagonists Ketamine or MK801 prevented RRHS induced lengthening of afterdischarges. Pretreatment with MK801 in a dose of 4mg/Kg simultaneously prevented lengthening of afterdischarges and loss of paired pulse inhibition.

The mechanisms behind kindling are unknown, however theoretical discussions of its pathophysiology focus on the balance between excitation and inhibition. Current and previously reported experiments demonstrate that NMDA receptor activation results in diminution of GABA mediated paired pulse inhibition which in turn mediates prolongation of afterdischarges observed in RRHS method of kindling.

452.5

DECREASE OF AMYGDALOID GABA-IMMUNOREACTIVE NEURONS AFTER KINDLING P.M. Callahan, J.M. Paris, K.A. Cunningham and P. Shinnick-Gallagher Dept. of Pharmacology, Univ. of Texas Medical Br., Galveston TX, 77550.

The inhibitory neurotransmitter GABA appears particularly sensitive to amygdala epileptogenesis. The present study was designed to investigate the in vivo kindling effects on GABA-immunoreactive (GABA-IR) neurons of the lateral and basolateral amygdaloid nucleus in the rat. Male Sprague-Dawley rats were anesthetized and implanted stereotaxically with bipolar stimulating electrodes in the basolateral nucleus. One week after surgical implantation, animals were electrically stimulated with cathodal pulses of 1 ms, 500 uA applied at 60 Hz for 1 s once per day until 3-5 stage 5 seizures were observed. The control group (N=4) consisted of non-stimulated implants and unimplanted rats. Kindled rats developed generalized seizures after an average of 14 days (range: 9-19; N=4). Histological recovery of the electrode tips indicated that placements were in or near the basolateral complex for all subjects. Tissue was processed for GABA on the contralateral side of the brain using avidin-biotin peroxidase immunohistochemical procedures. Within the lateral and basolateral complex, discrete brain sections (-2.12, -2.56, -3.14 and -3.60 mm from bregma, Paxinos & Watson, 1986) were chosen for quantification. The results indicate that, in comparison to controls, fully kindled animals showed a significant decrease in total number of GABA-IR neurons in the amygdala nuclei (ANOVA, $F_{(1,6)}=25.28$, $P<0.002$). Furthermore, GABA-IR neurons throughout each of the four coronal amygdala planes were reduced in kindled animals ($p < 0.05$). The present data suggest that electrical kindling of the amygdala results in a significant reduction of GABA-IR neurons in the lateral and basolateral areas throughout the contralateral amygdaloid nucleus. This reduction may be responsible for the observed loss of GABAergic IPSPs and the spontaneous epileptiform bursting which may ultimately contribute to amygdaloid epileptogenesis (Gean et al., 1989).

(Supported by NS24643 and DA05708)

452.6

DEVELOPMENT OF LONG-TERM SUBSENSITIVITY TO GABA IN DORSAL RAPHE NEURONS OF AMYGDALA KINDLED RATS.

T.D. Hernandez and D.W. Gallager. Dept. Psychiatry, Yale Univ. Sch. Med., New Haven, CT 06508.

Previously we reported a long-term change in neuronal sensitivity to GABA following amygdala kindling (Hernandez et al., Brain Res, 1990). Dorsal raphe neurons of amygdala kindled rats exhibited significant subsensitivity to GABA 4 weeks after the last fully generalized (Stage 5) seizure. This subsensitivity was equivalent in magnitude to that observed following chronic diazepam treatment; yet, unlike that produced by chronic diazepam treatment, subsensitivity due to amygdala kindling was not reversed by bath application of Ro 15-1788. We hypothesized that this alteration in GABA sensitivity might reflect neuronal changes corresponding to kindled seizure susceptibility and subsequent experiments have investigated this hypothesis.

The development of subsensitivity to GABA during the amygdala kindling process progresses through a continuum that reflects the Stage to which an animal has been kindled. That is, when measured 4 weeks after the last kindled seizure, dorsal raphe neurons are *supersensitive* to GABA following a Stage 2 seizure, *not different from controls* following a Stage 3 seizure and *subsensitive* to GABA following a Stage 5 seizure. In addition, subsensitivity to GABA appears to be permanent in that it is still measurable 3 months after the last Stage 5 seizure. This progression from GABA supersensitivity to subsensitvity parallels that observed following exposure to diazepam: acute diazepam exposure results in increased sensitivity to GABA, while chronic exposure (> 2 weeks) leads to subsensitivity. Unlike kindling, however, changes seen after benzodiazepine exposure return to control levels following drug discontinuation. Thus, amygdala kindling produces long-term, perhaps permanent, changes in neuronal sensitivity to GABA reflecting the Stage to which an animal has been kindled.

452.7

REGIONAL ANALYSIS OF GABA-STIMULATED CHLORIDE FLUX IN AMYGDALA KINDLED RATS. E.I. Tietz and T. H. Chiu. Dept. of Pharmacology, Medical College of Ohio, Toledo, OH 43699

GABA-stimulated chloride flux was evaluated in 4 brain regions (cortex, CTX; hippocampus, HIP; midbrain/brainstem, MBR and cerebellum; CER) of amygdala kindled rats. Male rats (n=16) were stimulated 2 X daily in the amygdala until 5 Stage 5 seizures were elicited. Control rats (n=16) were sham kindled. Microsacs, prepared from 4 pooled kindled or control rats 7 days after the last amygdala stimulation, were preincubated 15 min at 30°C. GABA (10 or 50 μM) and Cl^- (0.4 μCi) were added followed in 5 sec by rapid filtration and 2 buffer washes. Basal flux (nmoles mg protein) differed between regions but not between kindled and control groups (CTX, 17.7±.9 vs 17.6 ±1; HIP 21.5±1.1 vs 20.6±1; MBR, 30.6±1.8 vs 33.4±1.3; CER, 23.4±0.6 vs 26.5±1.0). There were significant ($p \leq .01$) decreases in net GABA-stimulated flux in CTX and HIP only at the lower GABA concentration. The decrease in flux in CER (9.7±.7 vs 6.4±.1) was not significant. There was no difference in MBR (3.1±.2 vs 2.9 ±.4). There was also no change in any brain region in the ability of 1 μM midazolam to stimulate 10 μM GABA-mediated flux. These results indicate long-lasting regionally specific changes in GABA complex function following amygdala kindling. The findings contrast those of decreased GABA-stimulated flux in brainstem of entorhinal-kindled rats and support those of decreased GABA complex function with chemical kindling. Supported by grants S07-RR05700 and R01-DA04075.

452.8

AUTORADIOGRAPHIC ANALYSIS OF ^{35}S-TBPS BINDING IN THE HIPPOCAMPUS OF KINDLED RATS. J.S. Petrasek, J.N. Nobrega, S.J. Kish and W.M. Burnham. Department of Pharmacology, University of Toronto, and Clarke Institute of Psychiatry, Toronto, Canada.

Previous studies have demonstrated that kindling induces a transitory increase in the binding of both GABA and benzodiazepine receptor ligands within the hippocampus (fascia dentata). We have now chosen to investigate whether a similar change in the binding of ^{35}S-TBPS to the "convulsant" site associated with the GABA-mediated chloride ionophore also occurs in the hippocampus of kindled rats. Subjects (n=7 per group) were kindled via stimulation of the entorhinal cortex and sacrificed with matched controls either 24 hours or 28 days after their sixth Stage 5 seizure. Binding of ^{35}S-TBPS (4 nM) was examined in multiple regions of the hippocampus in kindled and control brains via quantitative receptor autoradiography. No significant change in binding was observed after kindling in any of the hippocampal subdivisions examined, either 24 hours or 28 days after the last seizure. Our data suggest that kindling does not alter the number of chloride ionophore convulsant sites in the rat hippocampus, although it may alter the number of $GABA_A$ and benzodiazepine receptor sites. A detailed analysis of TBPS binding in other regions of the kindled brain is now in progress. (Supported by MRC Grant MA 5611.)

452.9

NORADRENERGIC COMPONENTS OF FOREBRAIN KINDLING: THE ROLE OF AMYGDALOID NOREPINEPHRINE. J Nierenberg, CD Applegate and JL Burchfiel. Comprehensive Epilepsy Program, University of Rochester School of Medicine, Rochester, NY 14642.

Forebrain depletion of norepinephrine (NE) facilitates kindled seizure development by reducing the number of trials spent in the early phase of kindling (seizure stages 1-2). We have hypothesized this result indicates the existence of a discrete NE-dependent transition from early to later (stages 3-5) kindling phases (Burchfiel & Applegate, Neurosci. Biobehav. Rev., 13:1989). In this study we have attempted to localize a NE-sensitive forebrain substrate which facilitates this transition. Rats were injected bilaterally into the amygdala with 6-hydroxydopamine (6-OHDA, 10 µg in 1 µl) prior to kindling from either amygdala (n=10) or septal nucleus (n=6). Amygdala-kindled rats with 6-OHDA lesions showed 40% reduction in the number of early, stage 1-2 trials (mean=6.7±1.6) relative to vehicle-injected controls (mean=11.5±1.0). Amygdala 6-OHDA also resulted in a reduced number of stage 1-2 trials in septal nucleus-kindled rats (mean=14.7±3.7) relative to uninjected controls (n=8, mean=22.0±3.9). These data suggest an involvement of amygdaloid NE in the kindling of forebrain structures. Ongoing experiments are being conducted to confirm whether the role of the amygdala is unique in this context or whether it is part of a limbic "loop" which modulates the NE-dependent transition from early to later stages of kindled seizure development.

452.10

AMYGDALA KINDLED SEIZURES AND BRAIN A_1 ADENOSINE RECEPTORS IN RATS. S.M. Anderson and D.D. Walczak*. Department of Medical Neurosciences, Walter Reed Army Institute of Research, Washington, D.C. 20307-5100.

In order to understand the development of post-traumatic epilepsy we are assessing changes in brain neurochemistry during various stages of seizure promotion using an electrical kindling model. The anticonvulsant properties of adenosine and evidence that upregulation of adenosine receptors is accompanied by reduced sensitivity to convulsants, prompted us to investigate the relationship between A_1 adenosine receptors and seizures. Male Sprague-Dawley rats were kindled from a bipolar depth electrode implanted in the left amygdala. Electrical stimulus was applied once every 24 hrs until either two consecutive stage 3 or three successive stage 5 seizures were achieved. EEG was recorded from the electrodes and the corresponding behavioral responses were scored using the rating scale of Racine. Three different control groups were studied at each seizure level: unoperated handled rats, sham-operated rats (implanted with electrodes but not stimulated), and a seizure control group, yolked to the kindled rats, which received suprathreshold stimulation (STS) induced seizures. Rats were decapitated 48 hrs after their last kindled or STS seizure and relevant brain regions were dissected from 2 mm coronal slices. A_1 adenosine receptors were measured in brain membrane homogenates using the selective adenosine analogue, [^{3}H]N^6-cyclohexyladenosine (CHA). We found no relationship between A_1 adenosine receptors and either the occurrence of STS convulsions or the development of seizures by kindling.

452.11

HIPPOCAMPAL KINDLING ENHANCES MEMBRANE-ASSOCIATED PROTEIN KINASE C IN THE BILATERAL HIPPOCAMPUS BUT NOT IN THE AMYGDALA/PYRIFORM CORTEX. A.Daigen*, K.Akiyama, T.Itoh* and S.Otsuki*. Dept. Neuropsychiatry, Okayama University Medical School, Okayama 700, Japan.

The effect of hippocampal (HIPP) kindling on protein kinase C (PKC) was investigated in the rat amygdala/pyriform cortex (AM/PC) and the right (contralateral) and left (ipsilateral) HIPP. Seven days after the last kindled seizure, there was no difference between the control and kindled group in cytosolic PKC activity in any part of the brain. In the kindked rats, a significant increase in membrane-associated PKC activity was found in the left HIPP (by 30%, $p<0.02$) and right HIPP (by 26%, $p<0.05$) as compared to the control. However, there was no significant alterations in the AM/PC. The protein concentrations in the crude cytosolic preparation did not differ between the two groups in any brain area examined, but those in the membrane preparations increased significantly in the left HIPP (by 22%, $p<0.02$) of the kindled rats. In the fraction co-eluted with PKC, significant increase in the protein concentration was confirmed in the ipsilateral HIPP for the cytosolic preparation as well as in the bilateral HIPP for the membrane preparation. These results suggest that activation of PKC may play an important role in the kindling mechanism.

452.12

KINDLING IN RATS: EVALUATION OF A HIGHLY SENSITIVE LOCUS IN THE POSTERIOR PART OF THE PIRIFORM CORTEX. W. Löscher, U. Wahnschaffe* and D. Hönack*.Dept. of Pharmacol.,Toxicol.& Pharmacy,. Sch.of Veterinary Medicine,3000 Hannover 71,FRG

The piriform cortex (PC), especially its deep anterior part (DPC), has been recently suggested to be a crucial epileptogenic site in the rat brain. We investigated the possible role of the posterior part of the PC (PPC) in electrical kindling. A locus in the deep cell layer (layer III) of the rostral portion of the PPC is described, which is considerably more sensitive to electrical stimulation than the DPC and the amygdala (AM). This locus can be readily kindled, and effects of antiepileptic drugs in PPC-kindled rats are similar to effects in AM-kindled rats, although the locus in the PPC tend to be more resistant to anticonvulsant effects. Unilateral lesions of the PPC significantly increase the seizure threshold of rats kindled from stimulation of the ipsilateral AM. The data suggest that the PPC locus described might represent a specific generating site within the PC.

452.13

PYRIFORM CORTEX INVOLVEMENT IN LIMBIC KINDLING. D.C. McIntyre and M.E. Kelly. Dept. of Psychology, Carleton Univ., Ottawa, Ont. K1S 5B6.

The role of the pyriform cortex (PC) in secondary generalization of kindled limbic seizures was investigated in 2 experiments. In the first experiment, rats previously kindled from the dorsal hippocampus (DH) and ipsilateral olfactory bulb (OB) were given systemic kainic acid (12 mg/kg, i.p.) to destroy the PC. Following this treatment, animals with complete loss of the PC exhibited strong hippocampal ADs when stimulated, yet were unable to re-develop secondarily generalized seizures. In addition, the electrographic and convulsive responses previously triggered by stimulation of the kindled OB were lost. In the second experiment, status epilepticus (SE) was induced via 60 min of stimulation to a kindled amygdala focus, producing complete PC damage in the kindled hemisphere. Kindling of the ipsilateral DH was then initiated. Subsequent seizure profiles of these animals suggested that the triggered DH seizure gained access to generalization mechanisms through the intact contralateral PC. Taken together these data suggest that the PC is extremely important, if not critical, to the generalization of kindled seizures from the dorsal hippocampus and olfactory bulb.

452.14

LONG LASTING DECREASE IN HIPPOCAMPAL MOSSY FIBER ZINC AFTER ENTORHINAL KINDLING IN RAT. DD Savage, DY Tso-Olivas* and JT Slevin. Dept. Pharmacology, Univ. New Mexico Sch. Med., Albuquerque, NM, 87131 and V.A. Research Service and Depts. Neurology and Pharmacology, Univ. Kentucky Coll. Med., Lexington, KY, 40536.

We have observed striking reductions in hippocampal formation (HPF) mossy fiber zinc in rats exhibiting kindling-induced spontaneous seizures. This observation prompted an investigation of whether mossy fiber zinc is reduced in rats 1 day or 4 weeks after two Class 5 kindled motor seizures.

Male Sprague-Dawley rats were assigned to one of three groups receiving either: 1) daily entorhinal kindling (EK) stimulations until they exhibited two successive Class 5 motor seizures, 2) daily electroshock (ECS) seizures on the last two days that an EK rat exhibited a Class 5 seizure or 3) a sham operation (electrode implanted unstimulated control). One day or 4 weeks after two successive Class 5 EK seizures, trios consisting of one EK rat, one ECS rat and one control rat were sacrificed and their brains frozen. Quantitative zinc:TS-Q fluorescence was measured in CA3 *stratum lucidum* in coronal sections of dorsal HPF and horizontal sections of ventral HPF.

Compared to control rats, mossy fiber zinc was reduced significantly in ventral but not dorsal HPF of ECS rats and in both dorsal and ventral HPF of EK rats 1 day after cessation of EK stimulations. The mossy fiber zinc reduction persisted in EK rats 4 weeks after EK stimulations. At this later time, mossy fiber zinc in the ECS group was not different from control.

The long lasting reduction in mossy fiber zinc in EK rats parallels the mossy fiber zinc reductions observed in kindling-induced spontaneously seizing rats as well as seizure-naive Genetically Epilepsy-Prone (GEPR-9) rats and alcohol withdrawal seizure prone (WSP) mice. This similarity in four different animal models of epilepsy suggests that a decrease in HPF mossy fiber zinc may be a contributing factor in the manifestation of a seizure prone state in genetically epileptic rodents and the persistence of enhanced seizure susceptibility in kindled rats.

452.15

DENTATE GRANULE CELL EVOKED RESPONSES DURING PROLONGED MAINTENANCE OF THE KINDLED STATE. L. J. Burdette, G. J. Hart* & L. M. Masukawa. Department of Neurology, Graduate Hospital, Philadelphia, PA 19146

Field responses recorded from dentate granule cells have been well characterized during the development of kindled seizures. To mimic more closely the human epileptic condition, we have extended these findings to observations following repeated generalized seizures. Rats were kindled by stimulation of the perforant path. Field responses were elicited by varying stimulus intensity, and by paired pulse and repetitive low frequency (.05 and 1 Hz) stimulation. Potentiation of the excitatory postsynaptic potential observed during kindling continued to increase during a 30 day rest, and did not change further once kindling stimulation was reinstated. The population spike was relatively unaffected throughout, except for an increase in threshold that was noted between 30-60 seizures. At this time, a second population response developed during repetitive low frequency stimulation. These secondary evoked potentials exhibit a latency of 80-120 ms following the triggered response, are intensity-dependent, and occur in the presence of significantly increased inhibition. The appearance of secondary responses has been noted reliably following a 48-72 hour seizure-free period, suggesting they are not immediate post-ictal sequelae. These results indicate that repetitive generalized seizures induce synchronous population discharges. It remains to be determined if these events ultimately contribute to seizure initiation.

453.1

PREFERENTIAL EXPRESSION OF SUPEROXIDE DISMUTASE GENE IN THE NEUROMELANIN-PIGMENTED NEURONS OF THE SUBSTANTIA NIGRA : IMPLICATIONS FOR PARKINSON'S DISEASE.

Zhang P.*, Ceballos* I(1), Hirsch E., Lafon M*., Sinet P. M*(1), Agid Y and F. Javoy-Agid.

INSERM U 289, Hôpital de la Salpêtrière 75013 PARIS, (1) URA CNRS 1335 - Laboratoire de Biochimie Génétique - Hôpital Necker Paris FRANCE

The dopaminergic neurons primarily affected in Parkinson's disease are the melanized neurons of the substantia nigra pars compacta. The involvement of oxygen free radicals has been considered as a potential cytotoxic cause of cell death. This lead to search for specific characteristics of toxic species defense system in these nigral neurons. Superoxide dismutase (SOD) which catalyses the conversion of superoxide radicals to hydrogen peroxide was examined.

The level of CuZn dependent SOD (CuZnSOD) gene expression was studied at cellular level in human mesencephalon post-mortem, by in situ hybridization using a 35-S-labelled cDNA probe homologous to human CuZnSOD mRNA. Labelled cells were densely distributed in the substantia nigra pars compacta, some cells were present in the ventral tegmental area. 95 % of the positively hybridized nigral cells were the large neuromelanin-containing cells, indicating CuZnSOD gene is preferentially expressed in the pigmented dopaminergic neurons. The high levels of CuZnSOD transcripts suggest the biochemical pathways leading to toxic oxygen species formation are active thus requiring a high CuZnSOD protein to facilitate removal of the toxic radicals. Alternatively, a high CuZnSOD activity might contribute to the neurodegenerative process itself.

The nigral melanized neurons represent a subset of dopaminergic cells with respect to their oxygen defense system. This may account for their preferential vulnerability in Parkinson's disease.

453.2

IRON LADEN NEURONS CHARACTERIZE SUBSTANTIA NIGRA RETICULATA AND GLOBUS PALLIDUS IN PARKINSON'S DISEASE. R. C. Switzer III and S. K. Campbell*. The R. H. Cole Neuroscience Lab., Dept. of Pathology, University of Tennessee Medical Center, Knoxville, TN 37920.

The identification of factors unique to the micro-environment of substantia nigra (SN) neurons in Parkinson's disease (PD) may provide valuable clues for understanding its etiology. One such factor may be iron since PD is one of a number of neurodegenerative diseases in which the areas principally affected form a subset of the iron-rich areas of the brain. Also, T2-weighted MRI shows differences in the iron-rich environment of SN in PD that suggest increased Fe or a change in its molecular matrix. In 14/14 cases of PD, 40u freeze-cut brain sections stained for ferric iron with the Perls method displayed a majority of the neurons as iron-positive (FeP) in the pars reticulata of SN and in both sectors of globus pallidus (GP). Neurons in other iron-rich areas did not display the cytoplasmic blue reaction product which had an appearance ranging from diffuse to densely packed granules. Some neurons contained both forms. The FeP neurons were clearly not ferruginated cells. FeP neurons were rare in age matched controls and cases of Alzheimer's disease. A preliminary examination of the compacta neurons with energy dispersive X-ray / EM shows that they also had anomalous iron. Two features distinguish the human SN from other species: 1) it has the highest concentration of iron, & 2) the compacta neurons share the iron-rich matrix of reticulata. Whatever the cause or defect, such excess iron poses a severe liability due to iron's capacity to generate cytotoxic free radicals. The examination of more cases will reveal if this phenomenon characterizes all PD cases or only a subset.

Supported by the Robert and Monica Cole Foundation.

453.3

CEREBRAL METABOLIC CHANGES DURING THE "ON-OFF" PHENOMENON IN PARKINSON' S DISEASE: A PET STUDY WITH APOMORPHINE. E. Broussolle*, P. Pollak*, L. Cinotti*, D. Le Bars*, G. Galy*, F. Lavenne*, C. Feuerstein*, F. Mauguière*, G. Chazot*. Dpt Neurology, Hôp. Antiquaille, Lyon; CERMEP, Hôp. Neurologique, Lyon; Dpt Clinic. & Biologic. Neurosci., CHU, Grenoble; France.

Long-term levodopa treatment of Parkinson' s disease (PD) provokes disabling motor fluctuations ("on-off" phenomenon). We measured regional cerebral glucose utilization during the "on" and the "off" motor phases by the (^{18}F)-fluorodeoxyglucose (FDG) method and positron emission tomography (PET) in 5 right-handed non-demented PD patients (4 males, 1 female; mean age ± SD: 60.2 ± 7.5 yrs; duration of disease: 12.2 ± 2.9 yrs; duration of "on-off" phenomenon: 4.1 ± 2.3 yrs). Patients had severe akinesia with no tremor during the "off" phase, and no or few dyskinesia during the "on" phase. Two PET scans were performed 48 hrs apart. Antiparkinsonian medications were discontinued the evening before each PET day. 15 min prior to FDG injection, patients (while in an "off" phase) received s.c. apomorphine (APO), a potent dopaminergic agonist, or the vehicle. The APO dosage (3-6.5 mg) was able to relieve akinesia within less than 15 min, and for a duration of about 1 hr. Patients were taking a peripheral dopaminergic antagonist, domperidone (60 mg/day orally), to block APO effects on cerebral blood vessels. Our results reveal that APO tends to reduce glucose utilization in the whole cerebral cortex (- 8.8 %; p = 0.08, NS; ANOVA) and in most of the 12 regions analyzed (- 5 to - 19 %; significant only in the temporal cortex, p = 0.05, and the cerebellar vermis, p < 0.01).

453.4

LEWY BODY NEUROFILAMENT EPITOPE ANALYSIS AND LOCALIZATION. W. D. Hill*, V. M.-Y. Lee*, H. I. Hurtig*, J. M. Murray*, and J. Q. Trojanowski. Dept. of Pathology and Laboratory Medicine, Univ. Pennsylvania Sch. of Med., Philadelphia, PA 19104.

Lewy bodies (LBs) are the pathological hallmark of Parkinson's disease (PD). This study sought to determine the extent to which each neurofilament (NF) subunit (L, M, and H) was present in LBs. A battery of 36 anti-NF antibodies, characterized as to subunit specificity and epitope domain, was employed to probe substantia nigral LBs from 15 PD cases. All 36 antibodies labelled LBs. The epitopes recognized by these antibodies included those in: the NF-L rod domain and C-terminus; the NF-M N-terminus, rod domain, sidearm, multiphosphorylation site (MPR), and C-terminus; and the NF-H rod domain and MPR site. The results showed that nearly the entire length of each subunit is present in LBs. Additionally, the staining pattern of the LBs suggested that the sidearms of NF-M and NF-H beyond the rod domain are altered or missing in the center of the LB core. In contrast, the N-terminus of NF-M, the C-terminus of NF-L, and the rod domain of all 3 subunits were present throughout the LB. Supported in part by AG09215 and AG05465.

453.5

STEP FORCE CHANGES IN PARKINSONIAN PATIENTS. F. Muller* and G.E. Stelmach. Motor Behavior Laboratory, University of Wisconsin, Madison, WI 53706.

Force production is an essential part of all motor acts. Previously, Parkinson's Disease patients (PD) have been shown to produce accurate isometric forces (Stelmach, G.E., Worringham, C.J., 1988), but with a higher variability (Stelmach, G.E., et al., 1989). Less interest had been shown for force termination problems in PDs. Eight PDs and eight age matched controls performed step changes of isometric pinch and elbow flexion forces. Starting from 30 % of maximum voluntary contraction level, the task was to increase, and/or decrease the force level by 15 and 30 % as fast and as accurately as possible.

The results showed that absolute peak force levels differed among and between groups. Reaction times indicated a slowing for force release compared to force increase tasks. The accuracy requirements affected finger and elbow joints differentially. Movement time showed a longer duration for release compared to complete release for groups and conditions, while the pinch force increase for normals was the same across pinch force amplitudes. For the pinch task, relative rate of force changes (first derivative) confirmed that the ability of force impulse scaling was impaired in PDs. The unusual accuracy requirements for elbow flexion, however, showed that normals and PDs adopted a movement time scaling strategy.

453.6

TEMPORAL-SPATIAL IRREGULARITIES IN PARKINSONIAN INDEX FINGER AGONIST/ANTAGONIST MUSCLE ACTIVITY. C.J. Hunker* and J.H. Abbs Dept. of Neurology, Speech and Motor Control Lab, Waiman Center, Univ. of Wisconsin, Madison, WI 53706.

Normal rapid, goal-directed limb movements are accomplished by regulating the intensity and timing of triphasic antagonistically acting muscles. A striking feature of Parkinson's disease (PD) is abnormally slow movements (**bradykinesia**). Temporal and amplitude scaling aberrations of triphasic muscle activity have been proposed as the pathological substrate for this clinical sign in the limbs. The intent of this study was to examine the pathophysiology of bradykinetic movements not typically mediated by the classical triphasic neural control strategy.

Index finger extension/flexion reversal movements of variable extent and intramuscular EMG activity from extensor digitorum, extensor indicis, and flexor digitorum superficialis muscles were recorded in PD and normal subjects. Movements about the MP joint (IP joints were splinted) were transduced using an electrogoniometer.

A linear relationship between peak velocity and movement amplitude was found in normals. In contrast, PD responses were variable; most were outside the normal 95% confidence interval. Phase plane discontinuities at various points along index finger extension trajectories characterized the bradykinetic movements, but could not be explained by gross EMG burst patterns. However, temporal-spatial irregularities in PD agonist/antagonist muscle activity may underly the abnormal phase plane relationships. (NIH Grant NS-132274)

453.7

ANTICIPATORY AND FEEDBACK POSTURAL RESPONSES IN PARKINSON'S DISEASE. •S.L. Glatt, M.E. Melnick, W. Koller, R. Hassanein*, J. Nash*, J. Redford*, G. Jayaraman*. Departments of Neurology, Physical Therapy, and Rehabilitation Medicine, University of Kansas Medical Center, Kansas City, KS 66103.

•The mechanism underlying postural instability in Parkinson's disease (PD) is poorly understood. Anticipatory postural responses precede or coincide with the planned action while subsequent corrections are feedback related. We studied the anticipatory postural response and feedback related response, by measuring the movement of the center of pressure (CP) with forward lifting of a 0.5 kg bar, in 37 PD patients; 16 stage 1 (PD1), 14 stage 2 (PD2) and 7 stage 3 (PD3) compared to 9 elderly (EC) and 15 young controls (YC). Maximal forward displacement, which reflects the deficit in anticipatory responses, was a mean for PD1 of 1.80 cm, PD2 1.96, PD3 2.91, EC 1.60 and YC 1.87. Average position of the CP, which is under feedback control, was for PD1 0.75, PD2 0.85, PD3 1.93, YC 1.19, and EC 0.75. There were differences ($p < .01$) between PD 3 and all other groups in the anticipatory responses and between PD3 and all except YC in the feedback response. Maximal mean CP position for PD3 (2.91 cm) was similar to the biomechanical calculation of CP for the barlift posture with arms outstretched (3.2 cm) without any compensatory responses. There was more impairment in anticipatory than feedback responses. We suggest that abnormalities in anticipatory postural responses are the cause of postural instability and falls in PD.

453.8

COGNITIVE AND MOTOR SEQUENCING IN PARKINSON'S DISEASE. W.W. Beatty and N. Monson*. Neuropsychiatric Research Institute, Fargo, ND 58107.

Previous reports indicate that patients with Parkinson's disease (PD) may have particular difficulty on tasks that require accurate sequencing in time or space. However, in all previous studies, the apparent sequencing deficit could also be attributed to impairment in memory, visual perception or motor function that occurs in PD.

In the present study we administered a very simple untimed test of cognitive sequencing (arranging pictures of highly familiar events in order) and a version of the Luria 3-step test of motor sequencing to 27 patients with idiopathic PD and 25 age-and education-matched normal controls. Both demented and nondemented PD patients displayed impairments on both sequencing measures. The extent of the patients' impairments in cognitive and motor sequencing were positively correlated, and the severity of deficits on both sequencing tasks was related to performance on the Wisconsin Card Sorting Test, but not to neurologic measures of disease severity. These results demonstrate the existence of a cognitive sequencing deficit in PD, and suggested that in PD both cognitive and motor sequencing difficulties may be related to dysfunction of circuits that involve the frontal lobes.

453.9

MOVEMENT SEQUENCING IN PARKINSON'S DISEASE. E. A. Roy, J. Saint-Cyr, A. Taylor & A. Lang*. Movement Disorders Clinic and Department of Psychology, Toronto Western Hospital, Toronto, Ontario, Canada M5T 2S8.

The purpose of this study was to examine movement sequencing in Parkinson's disease (PD). Fifteen PD patients and five age-matched normals were required to learn a sequence of 3 or 4 hand movements on a sequencing board. Each trial involved two phases, a perceptual phase in which pictures depicting the movements were present and, if the patient performed correctly, a memory phase in which he attempted to perform the sequence from memory without the aid of the pictures. The criterion for learning was 5 consecutive correct trials in the memory phase. Videotaped performance was examined in terms of trials to reach criterion, errors (sequencing errors and perseverations), total response time and its components, time for each response element and time between each response. There were no group differences in trials to criterion or errors, but marked differences in response timing were apparent. Total response time was significantly longer for the PD patients which was due to both increased time to make each response as well as increased interresponse times. The relative timing reflected in the proportion of the total time spent in each component of the sequence was also significantly different for the two groups. The implications of these findings for understanding the movement sequencing impairments in PD are discussed. (Supported by a grant from The Parkinson's Foundation of Canada.)

453.10

PLANNING AND SPATIAL WORKING MEMORY IN PARKINSON'S DISEASE. A.M.Owen*, M.Galton*, P.N.Leigh, C.D.Marsden*, N.Quinn*, B.J.Sahakian, B.Summers* & T.W.Robbins. Institutes of Psychiatry and Neurology, London, U.K. and Department of Experimental Psychology, University of Cambridge, Cambridge, U.K.

Groups of patients with idiopathic Parkinson's disease, either medicated or unmedicated, were compared with a large sample of normal controls on a computerised battery of tests designed to investigate the cognitive processes involved in 'planning'. In a series of problems based on the 'Tower of London' test, a group of medicated patients were shown to be impaired in the amount of time spent thinking about (planning) the solution to each problem. Additionally, an impairment in terms of the number of moves required to reach a solution on this test was only evident in those patients 'later in the course' of the disease and was accompanied by a deficit in associated tests of spatial working memory and spatial span. In contrast, a group of patients who were unmedicated and 'early in the course' of the disease were unimpaired in all respects compared to normal controls.

These data are compared to those from a group of young neuro-surgical patients with localised excisions of the frontal lobes and are discussed in terms of the progressive nature of the cognitive deficit in Parkinson's disease.

453.11

PARKINSON'S DISEASE AND FLUCTUATIONS IN LEVODOPA DO NOT IMPAIR EXPLICIT VISUAL LEARNING. J. Doyon, D. Côté*, A. Pelletier*, G. Brouillette*. Ecole de Psychologie, Université Laval, Ste-Foy, Qc, Canada, G1K 7P4.

Recognition tasks have shown to be very useful for studying memory functions in Parkinson's disease (PD) because they do not require the participation of the motor system. Such paradigm was employed in the present study to evaluate the effects of PD and of fluctuations in levels of levodopa on explicit visual learning. The performance of 10 non-demented PD patients, both during levodopa stimulated and unstimulated states (i.e., approximately 16 hours after the last drug intake) was compared to that of 10 matched healthy controls on the visual paired associates subtest of the Wechsler Memory Scale-Revised. A new version of this task was also created to eliminate any practice effect between the two testing sessions. These tasks were administered on separate days in a counterbalanced order. The results of the control subjects showed that the two versions of the visual learning task were equivalent. The performance of the parkinsonian patients on this task did not differ significantly from that of the controls. Furthermore, the decreased in the level of levodopa did not affect their learning ability, although it produced significant changes in their motor status. Thus, these results suggest that Parkinson's disease and variations in levels of levodopa do not impair explicit visual learning.

453.12

LOSS OF SELECTIVE MAO-B INHIBITION WITH LONG-TERM ADMINISTRATION OF L-DEPRENYL IN RODENTS: CROSSOVER TO MAO-A INHIBITION AND CLINICAL CONSIDERATIONS. I.A. Terleckyj, B.A. Sieber, and R.E. Heikkila. Dept. of Neurology, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ 08854.

The preliminary evaluation of patients with Parkinson's Disease (PD) in the DATATOP study has shown that l-deprenyl (Selegiline), at a dose of 5-10 mg daily, has been efficacious in delaying the need for therapy with l-dopa. In rats and mice, single doses of 2 mg/kg of l-deprenyl selectively and completely inhibit the B form of monoamine oxidase (MAO), with no significant inhibition of MAO-A activity. However, in the present study with l-deprenyl and other selective, irreversible MAO-B inhibitors (MDL 72145, AGN 1133), the daily administration of clinically relevant doses (0.2 to 2.0 mg/kg) to mice and rats for up to 4 weeks led to a loss in selectivity of MAO-B inhibition, with extensive inhibition of MAO-A occurring in both the brain and periphery. Interestingly, when rodents were given 2 mg/kg of clorgyline, a highly selective MAO-A inhibitor, for the same length of time no crossover to B inhibition was seen. It is possible that long-term administration of l-deprenyl to PD patients also leads to MAO-A inhibition, raising the possibility that these patients may be susceptible to hypertension after ingesting foods containing tyramine (the cheese effect). This correlates with other work done in normal humans showing that daily doses of l-deprenyl increase the pressor effects to a tyramine challenge (Clin. Pharm. & Ther. 46: 1989). Moreover, l-deprenyl is extensively metabolized to l-methamphetamine and l-amphetamine, both of which act as CNS stimulants. All of these observations suggest that the effects of l-deprenyl may not be due solely to its inhibition of MAO-B activity.

453.13

(-)-DEPRENYL AND DOPAMINE TRANSMISSION: ELECTROPHYSIOLOGICAL RECORDINGS IN THE RAT CAUDATE NUCLEUS. I.A. Paterson, M.D. Berry and A.V. Juorio, Neuropsychiatric Research Unit, University of Saskatchewan, Saskatoon, Saskatchewan, S7N 0W0.

(-)-Deprenyl is a monoamine oxidase type B (MAO-B) inhibitor with antiparkinsonian actions. It has been postulated that (-)-deprenyl will exert its antiparkinsonian actions by inhibiting the oxidative deamination of dopamine (DA), thus potentiating DA neurotransmission. In the rat striatum, however, doses of (-)-deprenyl which specifically inhibit MAO-B do not affect DA catabolism but rapidly increase the levels of 2-phenylethylamine (PE) (Juorio & Paterson, (-)-Deprenyl and dopamine transmission: lack of effect on striatal dopamine metabolism, this meeting). Single-unit recordings of both spontaneously active and glutamate-driven cells were made in the dorsal caudate nucleus of urethane-anaesthetised male Wistar rats. Iontophoretic applications of R(-) apomorphine (a mixed D1/D2 agonist) and (±) PPHT (a D2 agonist) consistently inhibited the firing of caudate neurones. Iontophoretic and intra-arterial injection (30-100 μg/kg) of PE potentiated the neuronal responses to the DA agonists, reducing the IT_{50} of the responses. (-)-Deprenyl (2 mg/kg, i.p.) potentiated the neuronal responses to apomorphine and PPHT within 15-30 minutes of injection. Studies on the effects of (-)-deprenyl on neuronal responses to SKF-38393 (a D1 agonist) are in progress. These results demonstrate the (-)-deprenyl does potentiate DA transmission but suggest that it does so indirectly by increasing brain levels of PE, an amine which potentiates neuronal responses to DA and DA agonists.

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453.14

(-)-DEPRENYL AND DOPAMINE TRANSMISSION: LACK OF EFFECT ON STRIATAL DOPAMINE METABOLISM. A.V. Juorio and I. A. Paterson, Neuropsychiatric Research Unit, M.R. Bldg, University of Saskatchewan, Saskatoon, Sask., Canada. S7N 0W0

(-)-Deprenyl is a specific monoamine oxidase type B (MAO-B) inhibitor which has been used as an adjuvant to L-DOPA therapy because it lowered the daily requirement for L-DOPA and its side effects. Recently, it has been shown that (-)-deprenyl delays the progression of Parkinson's disease in the earlier stages of the disease. It has been assumed that (-)-deprenyl acts mainly by inhibiting the oxidative deamination of dopamine (DA), however, acute administration of (-)-deprenyl does not affect DA metabolism. We propose that the acute action of (-)-deprenyl may involve 2-phenylethylamine (PE), a putative neuromodulator of DA transmission. PE may coexist with DA in the nigro-striatal projection as 6-hydroxydopamine lesions or electrical stimulation of the nigra alters its levels in the striatum. PE is not stored in reserpine-sensitive granules and is released by diffusion down a steady-state concentration gradient between the terminal and glial cells where it is metabolized by MAO-B. In rats, (-)-deprenyl (0.5-4 mg kg^{-1}, i.p.) does not change striatal levels of DA, DOPAC and HVA but increases PE levels (160-350 % of controls). The effects were observed within 1 hour after acute administration of (-)-deprenyl. In electrophysiological studies, (-)-deprenyl (2 mg kg^{-1}) potentiates caudate neurone responses to DA agonists, an effect that is similar to the actions of PE (Paterson et al., (-)-Deprenyl and dopamine transmission: electrophysiological recordings in rat caudate nucleus, this meeting). Taken together, it appears that (-)-deprenyl may increase brain PE levels, resulting in a potentiation of DA transmission.

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453.15

L-DOPA ACCUMULATES SELECTIVELY IN THE DOPAMINE DEPLETED STRIATUM FOLLOWING CHRONIC TREATMENT. Robert J. Carey Department of Psychiatry SUNY and VA Medical Centers, Syracuse, NY 13210

Rats with unilateral 6-hydroxydopamine lesions were treated with L-DOPA (10 or 20 mg/kg IP) plus Carbidopa (1.0 or 2.0 mg/kg IP). The effects of L-DOPA on Dopamine and L-DOPA metabolism were assessed after acute and after chronic daily injections for up to 2-months. Acute L-DOPA injections induced dose dependent increases in L-DOPA and methyl DOPA in limbic and striatal tissues which were equivalent for the intact and dopamine deficient hemispheres. Parallel increases were found for the dopamine metabolites, DOPAC, HVA and 3MT. Chronic L-DOPA treatment induced a three-fold increase in L-DOPA in the dopamine denervated striatum as compared to the intact striatum. This three-fold increase was found for both the 10 and 20 mg/kg L-DOPA treatments. No differences were observed in terms of methyl DOPA levels between the intact vs. the dopamine depleted striatum. In limbic tissue, there were no differences between intact and dopamine depleted hemispheres in either L-DOPA or methyl DOPA levels. The finding of a selective accumulation of L-DOPA in the dopamine depeleted striatum would appear to have implications for the changes in clinical efficacy that occur with chronic L-DOPA treatment of Parkinson's disease.

453.16

IRREVERSIBLE INHIBITION OF DOPAMINE UPTAKE: A NOVEL TREATMENT FOR PARKINSON'S DISEASE. A. Jewell-Smith, S.T. Buxton and L.P. Dwoskin. Div. Pharm. Exp. Ther. Col. Pharmacy, Univ. Kentucky, Lexington, KY 40536.

Reuptake of dopamine (DA) into the presynaptic terminal is the primary means of DA removal from the synaptic cleft. Irreversible blockade of reuptake theoretically would produce a greater synaptic concentration of DA in the cleft upon neuronal firing. This novel approach to the therapeutics of Parkinson's disease may extend the period of normal function in these patients. We recently reported (Dwoskin, et al., Biochem. Pharm. 38: 549, 1989) that DSP4 irreversibly blocks the DA uptake site, indicated by a decreased density with no change in affinity of [3H]mazindol binding sites in rat striatal membranes and a decrease in [3H]DA accumulation by striatal slices. In the present study, in vitro exposure of slices to DSP4 resulted in a decrease in the field-stimulation evoked overflow of endogenous dihydroxyphenylacetic acid (DOPAC). The EC50 was 5 uM. Basal outflow of DOPAC was not altered, indicating a selective effect on evoked release. Therefore, after exposure to DSP4, the DA which was released may have been unable to enter the terminal to be metabolized to DOPAC. Alternatively, the DSP4-induced decrease in DOPAC may be due to a diminished release of DA, since DA was not detected in superfusate, unless nomifensine was present in the superfusion buffer. (Supported by NIH BRSG S07 RR05857-08).

453.17

N-0923, A SELECTIVE DOPAMINE D2 RECEPTOR AGONIST, IS EFFICACIOUS IN RAT AND MONKEY MODELS OF PARKINSON'S DISEASE. J.D. Belluzzi, E.F. Domino+, J.M. May* and D.A. McAfee, Whitby Research Inc., Irvine, CA 92715 and +Department of Pharmacology, University of Michigan, Ann Arbor, MI 48109.

Certain aminotetralins are known to be potent dopamine D2 receptor agonists (Horn, *Drugs of the Future*, **12**:220, 1987). N-0923, (-)2-(N-propyl-N-2-thienylethylamino)-5-hydroxy-tetralin, recognized the high and low affinity states of the D2 receptor in membranes from bovine caudate with a K_{low} = 79 nM. Selectivity was D2/D1 = 15 and D2/α_2 = 1.4. N-0923 inhibits dopamine uptake, prolactin secretion and the α_2 receptor.

N-0923 (3-300 nmol/kg, sc) induced dose-dependent contralateral turning behavior in rats with unilateral 6-OH-dopamine lesions of the substantia nigra. The ED_{50} of 30 nmol/kg was effective for 1 hr. The positive enantiomer (N-0924; 300 nmol/kg, sc) was without effect.

A hemiparkinsonian syndrome was induced in 4 *Macaca nemestrina* monkeys by unilateral infusion of the neurotoxin MPTP into the right carotid artery (Bankiewicz, *et al., Life Sci.* **39**:7, 1986). Video recordings of free-moving behavior revealed bradykinesia, disuse of the contralateral upper limb and ipsilateral turning behavior. N-0923 (3-300 nmol/kg, im) induced contralateral turning behavior, exploratory activity and contralateral limb usage. The ED_{50} for turning (30 nmol/kg) was effective for 0.5 hr. The potency order for induction of contralateral rotations was PHNO > N-0923 > bromocriptine. N-0924 (300 nmol/kg, im) was ineffective. We conclude that N-0923 may be useful as a therapeutic agent in the treatment of Parkinson's disease.

453.18

EFFECT OF ADDITION OF CY 208-243 TO CHRONIC BROMOCRIPTINE TREATMENT ON BRAIN DOPAMINE RECEPTORS IN MPTP MONKEYS. C. Gagnon[1], B. Gomez Mancilla[2], P.J. Bédard[2] and T. Di Paolo[1] [1]School of Pharmacy, Laval Univ. and Dept of Molecular Endocrinology, CHUL, Quebec G1V 4G2 and [2]Dept of Anatomy, Fac. Med. , Laval University, Quebec G1K 7P4, Canada

We have compared the effect of chronic treatment of MPTP monkeys with bromocriptine (BRC) (D-2 agonist) alone or in combination with CY 208-243 (D-1 agonist). D-1 and D-2 receptors were quantified by autoradiography of [^{3}H]-SCH 23390 (D-1 antagonist), [^{3}H]-spiperone (D-2 antagonist), [^{3}H]-SKF 38393 (D-1 agonist) and [^{3}H]-N-propylnorapomorphine (D-2 agonist) binding in the right striatum of these animals. Binding on homogenates (left striatum) was also performed for D-1 and D-2 antagonist sites. MPTP monkeys had a decrease of DA of more than 95% vs control as measured by HPLC. D-1 and D-2 antagonist as well as agonist sites were increased following MPTP. Both DA treatments decreased the supersensitivity of D-1 and D-2 antagonist and agonist sites. The affinity of the D-1 antagonist site was increased following both DA treatments compared to control and MPTP animals. The addition of CY 208-243 to the BRC treatment was without effect on D-1 and D-2 agonist and antagonist sites compared to the treatment with BRC alone. Changes in agonist and antagonist sites may explain behavioral recovery seen after both DA treatments. Supported by the Parkinson Foundation of Canada and the MRC.

453.19

GM1 GANGLIOSIDE PROMOTES RECOVERY FROM MPTP-INDUCED DAMAGE IN THE DOPAMINE SYSTEMS OF MONKEYS AND CATS AND IN CULTURES OF RAT DOPAMINE NEURONS. J.S. Schneider and L. Iacovitti. Dept. of Neurology, Hahnemann University School of Medicine, Philadelphia, PA. 19102.

In primates and cats, MPTP produces loss of ventral mesencephalic dopamine (DA) neurons (particularly in substantia nigra pars compacta, with relative sparing in other adjacent areas), loss of striatal DA, and some impairment in motor function. Cultures of dissociated embryonic rat (E15) mesencephalon, exposed to MPP+, also show loss of some DA neurons. Most surviving DA neurons have structural damage (shrunken cell bodies, short, fragmented processes). The present study examines the extent to which GM1 ganglioside treatment can reverse the effects of MPTP/MPP+ *in vivo* and *in vitro* and whether GM1 can protect cultured neurons from toxin-induced damage. In cats given 7 days of MPTP and made severely parkinsonian, GM1 treatment (30 mg/kg daily for 6 wks.) significantly increased DA and metabolite levels in the caudate nucleus (85% DA increase after GM1), however, these animals still had 95% loss of caudate DA. Cats which received less MPTP (3-5 days) had less severe dorsal striatal DA loss (90%), less severe damage to ventral mesencephalic DA neurons and had striatal DA levels brought closer to normal (only 75% depletion) with GM1 treatment. Similar results have been obtained with MPTP-treated squirrel monkeys. All GM1-treated monkeys had increased striatal DA levels, with the greatest increases in less severely affected animals. Pre-treatment of rat mesencephalon cultures with GM1 (50 uM) failed to protect against MPP+-induced damage. However, cultures treated with GM1 (50 uM, 24 hrs.) after MPP+ (10uM, 48 hrs.) looked healthier than control cultures and contained DA neurons with numerous finely beaded processes which made extensive plexuses. These results suggest that GM1 has definite reparative effects on injured dopamine neurons in relevant *in vivo* models and stimulates repair and sprouting in injured DA neurons in culture. Supported by the Amer. Parkinson's Dis. Assoc.

NEUROTOXICITY: AMINO ACIDS

454.1

Effects of methamphetamine and MK-801 on dopamine in the striatum of awake rats as measured by in vivo brain microdialysis. F.B. Weihmuller, S.J. O'Dell, B.N. Cole*, and J.F. Marshall, Dept. of Psychobiology, University of California, Irvine, CA 92717.

Although the NMDA receptor antagonist MK-801 attenuates methamphetamine (MA)-induced striatal dopamine (DA) neurotoxicity, the mechanism underlying this protection is unknown. We used in vivo microdialysis in awake rats to determine the effects of MK-801 and MA on the levels of extracellular striatal DA (and metabolites) and behavior. A single injection of MA (6.25 mg/kg, sc) induced a prolonged increase (> 6 hrs) in extracellular DA (an 8-fold peak response), reduced DOPAC to 30% of basal levels, and increased sniffing, circling, and locomotion. Given alone, MK-801 (0.5 mg/kg, ip) also produced an increase in extracellular striatal DA (a 2-fold peak response, shorter in duration than that with MA), and increased sniffing and locomotion. In contrast to MA, MK-801 increased extracellular DOPAC and caused head-weaving and hindlimb ataxia. Pretreatment with MK-801 markedly attenuated the MA-induced DA increase and increased rather than decreased DOPAC. Despite its attenuation of MA-induced DA increases, MK-801 potentiated some components of MA-induced behaviors and increased their duration. These findings suggest that although MK-801 alone increases extracellular striatal DA, it reduces the MA-induced striatal DA overflow, which may help explain its attenuation of MA-induced dopaminergic terminal injury.

454.2

INTERACTIONS OF MK-801 WITH GLUTAMATE- AND METHAMPHETAMINE-EVOKED ^{3}H-DOPAMINE RELEASE FROM STRIATAL SLICE. J.F. Bowyer, R.R. Holson, S.F. Ali, and W. Slikker, Jr.* Div. of Reprod. & Develop. Tox., Natl. Ctr. for Tox. Res., Jefferson, AR 72079.

MK-801 effects on L-glutamate (GLU)- and methamphetamine (METH)-evoked release of ^{3}H-dopamine (DA) from striatal slices in untreated and rats treated with toxic doses of METH (5 mg/kg x 4 doses/at 2 hr intervals) were evaluated. Without Mg^{2+} present, 40 µM and 1 mM GLU evoked ^{3}H-DA released (5% or 14% of total ^{3}H-DA stores [TS] over 10 min, respectively) from striatal slices of untreated rats. GLU-evoked release was inhibited by MK-801>>PCP> (+)SKF 10,047. Two weeks after in vivo METH, GLU-evoked release (no Mg^{2+} present) was decreased. With 1.25 mM Mg^{2+} present, 10 mM GLU evoked a slight release of ^{3}H-DA (2.1% TS) which was increased to 4.2% TS when 10 µM nomifensine was present. Release with nomifensine present could be inhibited 35% by 5 µM MK-801. With or without 1.25 mM Mg^{2+}, 0.5 and 5 µM METH evoked a release of ^{3}H-DA (7% and 20% TS over 10 min, respectively) which was additive with GLU-evoked release. MK-801 (5µM) did not affect METH-evoked ^{3}H-DA release with or without Mg^{2+} present. These results indicate that MK-801 protection against METH toxicity is not via presynaptic inhibition of METH-evoked DA release. However, since presynaptic release of DA by METH and GLU is additive; MK-801 might reduce GLU-mediated DA release during METH exposure.

454.3

EFFECTS OF HIGH-DOSE METHAMPHETAMINE (MA) ON NOREPINEPHRINE (NE) & DOPAMINE (DA) UPTAKE SITES IN RAT BRAIN MEASURED BY QUANTITATIVE AUTORADIOGRAPHY. D.J. Brunswick, S.M. Tejani-Butt & M. Hauptmann*. Dept. Vet. Affairs Med. Ctr. & Univ. of Pa Sch. of Med., Phila., PA 19104.

It has been reported that high doses of MA cause neurotoxic effects on serotonin (5-HT) and DA neurons in brain. However, NE neurons have been thought to be spared from neurotoxic effects. In addition, the neurotoxic effect of MA on DA uptake sites have previously been examined only in striatum. We have examined the effects of MA on NE and DA uptake sites in rat brain by quantitative autoradiography using ^{3}H-nisoxetine (Tejani-Butt et al., this meeting) or ^{3}H-mazindol, respectively. Rats were treated with either MA (15mg/kg free base, n=10) or saline (n=6) given s.c. every 6 hrs for 5 doses and killed 7 days following the last dose. Areas examined were from brain slices taken at plates 30 and 57 of the atlas of Paxinos and Watson (1986) for NE uptake sites and plates 13 and 37 for DA uptake sites. MA caused significant reductions (17-33%) in NE binding in basolateral and lateral amygdaloid nuclei and dorsomedial hypothalamus. No reductions were seen in any other area, including locus coeruleus, a cell body area. DA uptake sites were decreased by 23-33% in DA terminal areas - caudate putamen, nucleus accumbens and olfactory tubercle; in contrast, they were unaffected in DA cell body areas - ventral tegmental area and substantia nigra. We have previously shown that MA is neurotoxic in 5-HT terminal areas, but not in 5-HT cell body areas (*Brain Res.*, 505:123, 1989). We conclude that 1) MA may be neurotoxic to NE neurons in specific brain areas, and 2) neurotoxicity is limited to terminal field areas for all three monoamines. **(Supported by research funds from the Department of Veterans Affairs & USPHS grant DA 05317).**

454.4

BIPHASIC RELEASE OF DOPAMINE (DA) FROM CORPUS STRIATUM (CS) IN VITRO AND ELEVATION OF AMINE NEUROTRANSMITTER METABOLITES IN CEREBROSPINAL FLUID (CSF) OF PUSH-PULL PERFUSED RATS BY AMMONIA. S.Kuhananthan, V.D.Ramirez and R.V.Barresi*. Dept. of Physiology, University of Illinois, Urbana, IL 61801.

Ammonium chloride (0.5-12mM) releases dopamine (DA) and DOPAC in a biphasic manner from fragments of rat CS (n=4). The first phase reaches plateau at 3mM, when the DA release (pg/mg/40min., mean±SEM) reaches 2340±238 and the second phase reaches plateau at 7.5mM, when the DA release reaches 5450±1870. The first phase was associated with a parallel increase in DOPAC and was inhibited by tetrodotoxin (DA release, 841±26), a sodium channel blocker and D-600 (DA release, 375±123), a calcium channel blocker, but not by nomifensine (DA release, 1621±181), a DA uptake blocker. The second phase (DA release; second plateau-first plateau = 3110±698) was not associated by an increase in DOPAC and occurred to a significant extent in the presence of D-600 (DA release, 2150±388). This phase was inhibited by nomifensine (DA release, 809±277), but not by tetrodotoxin (DA release, 5320±891). It is postulated that the first phase is due to selective abolition of IPSP, a known action of ammonia. The second phase may be due to leakage of cytoplasmic DA using the uptake carrier as reversal of transport, analogous to the action of amphetamine. Preliminary results show that intraperitoneal (i.p) injection of 30mg/kg NH_4Cl produces 20 fold elevation in the CSF concentrations of DOPAC and 5-HIAA, in push-pull perfused rats in the lateral ventricle. These high levels last for 2-3h and did not occur in control rats infused with i.p saline. Ammonia concentrations above 1mM are found in hepatic coma and the release of amine neurotransmitters may be responsible in the pathogenesis.

454.5

COMPARISON OF METHAMPHETAMINE-INDUCED NEUROTOXICITY IN VARIOUS STRAINS OF MICE K.J. Takacs, D.E. Vitagliano*, P.K. Sonsalla and R.E. Heikkila Dept. of Neurology, UMDNJ-Robert Wood Johnson Med. Sch., Piscataway, NJ 08854

Methamphetamine (METH) administered to mice in repeated high doses is selectively neurotoxic to dopaminergic neurons in the neostriatum. This neurotoxicity is dependent upon the METH-induced release of dopamine. The extent of damage caused by a given dose of METH, which is assessed by decrements in neostriatal dopamine content and tyrosine hydroxylase activity, varies among different strains of mice. For example, in the present study we found that CFW mice were substantially more sensitive to the neurotoxicity induced by METH than were C57bl mice; both strains came from Charles River Breeding Laboratories. A lesser but still significant difference was observed between two strains obtained from Jackson Laboratories; SWR/J mice were more sensitive to METH-induced neurotoxicity than were CBA/J mice. In experiments done with neostriatal tissue slices obtained from untreated mice of the four strains, there were no pronounced differences in the capacity of METH to cause dopamine release. Our laboratory previously demonstrated that MK-801, a non-competitive NMDA receptor antagonist, was able to attenuate METH-induced neurotoxicity, suggesting that glutamate plays a role in mediating the damage. Interestingly, the CBA/J mice are more sensitive than are the SWR/J mice to the stimulatory and lethal effects of the methylxanthines, which are adenosine receptor antagonists. These strains also have different numbers of adenosine receptors. It is possible that variations in the interactions between or among dopamine/glutamate/adenosine may contribute to the observed differences in METH-induced neurotoxicity.

454.6

ASSESSMENT OF RAT'S BEHAVIOR IN A RADIAL ARM MAZE DURING EXPOSURE TO MAGNETIC FIELDS. RH Lovely, JA Creim, DL Miller and LE Anderson. Battelle, Pacific Northwest Laboratory, Richland, WA 99352.

This study is to determine if the *in vitro* efflux of calcium ions from animal cortex, that results from exposure to ELF fields, is physiologically significant *in vivo*. Long term potentiation (LTP) and memory in the rat, when performing in a radial arm maze (RAM), have been shown to be casually dependent on movement of free calcium ions in animal cortex. Both effects rely on glutamate binding to the NMDA receptor which in turn causes conformational changes in the Ca^{2+} ion ionophore. The effects and the resulting Ca^{2+} ion current are necessary conditions for LTP and RAM memory in rats. We are assessing RAM performance in rats while they are exposed to ELF and dc magnetic fields (MFs). The exposure system produces uniform (+/- 5%) ELF and dc MFs (vertically and horizontally) within the RAM. The field strengths are a dc MF of 2.6×10^{-5} T (0.26 G) in combination with a 60-Hz MF of 5×10^{-5} T*rms* (0.5 G). Briefly, the RAM consists of eight equal length arms radiating out from a central arena with a door at the entrance and a food cup at the end of each arm. Twenty-four male Sprague Dawley rats were food deprived to 80%-85% of their free feeding weight and then individually assessed in the RAM daily. Twelve rats were exposed during the RAM assessments, while the other 12 rats were sham-exposed. Presently the study is ongoing, but preliminary analysis of errors/group using a repeated measures ANOVA approach significance ($p<0.086$), with the exposed group making more errors. This data, when combined with results of a similar previous study, indicate a significant increase in errors made by the exposed groups ($p<.015$). Work supported by DOE/OESD under Contract DE-AC06-76RLO-1830.

454.7

ROLE OF GANGLIOSIDES AND Ca^{2+}-DEPENDENT ENZYMES IN GLUTAMATE NEUROTOXICITY. H.Manev, A.Guidotti, R.Siman§, and E.Costa. FGIN, Georgetown University, Washington, D.C. 20007; §Cephalon, Inc., West Chester, PA 19380.

The delayed neuronal death, that ensues after abusive stimulation of glutamate receptors, is a Ca^{2+}-dependent process that can be prevented by increasing the neuronal membrane content of natural gangliosides (GM1, GT1b) or by the application of their synthetic derivatives (LIGA4, LIGA20) (J.Pharmacol.Exp.Ther.,1990,252:419). We studied, in primary cultures of rat cerebellar granule neurons, the influence of these sphingolipids on glutamate-induced: (a) activation of Ca^{2+}-dependent cysteine proteinase (calpain) by quantitative immunoblotting of spectrin proteolytic fragments, (b) membrane translocation of protein kinase C (PKC) by immunoblotting and 3H-phorbol ester binding, (c) neuronal death. Calpain activity was dependent on micro-molar concentrations of Ca^{2+} (calpain I) in neuronal, and on millimolar concentrations (calpain II) in glial cultures. Glutamate induced a dose-dependent activation of calpain I and a translocation of PKC only in neuronal cultures. These changes outlasted the removal of a toxic dose of glutamate from the culture and preceded neuronal death. Pretreatment with GM1 or LIGA4 prevented the translocation of PKC, the prolonged activation of calpain I, and neuronal death. In vitro experiments excluded a direct inhibitory action of sphingolipids on calpain I. The prolonged translocation of PKC may be operative in maintaining the increased calpain I activity elicited by neurotoxic doses of glutamate.

454.8

OPTIMIZATION OF CONDITIONS FOR SEPARATION OF TEN TRYPTOPHAN METABOLITES BY RP-HPLC. C-Z. Chuang (1,2), F.A. Ragan, Jr. (1,2) and C. Prasad (1,3) Lab. of Neurosciences (1), Pennington Biomedical Research Center; Departments of Pathology (2) and Medicine (3) (Endocrinology Section), LSU Medical Center, New Orleans, LA 70112.

Tryptophan (TRP) hydroxylase and TRP oxygenase (TO) are two major pathways of TRP metabolism in brain. The TO pathway yields many interesting neuroactive metabolites: quinolinic acid, kynurenic acid, kynurenine and 3-hydroxy-kynurenine. Studies into the presence and the roles of these and other potential TRP metabolites (anthranilic, 3-hydroxyanthranilic, xanthurenic, quinaldic, and picolinic acids) in brain are limited by our inability to measure low levels of multiple metabolites in a single biological sample. Therefore, a RP-HPLC method for the separation of ten TO metabolites has been developed by sequential optimization of mobile phase pH, triethylamine concentration and gradient elution. Resolution obtained is very good with an analysis time, including re-equilibration period, of less than 30 min. To our best knowledge this is the first RP-HPLC method that separates TRP and ten metabolites of TO pathway in a single chromatographic run.

454.9

TRIMETHYLTIN VERSUS KAINIC ACID TOXICITY: DIFFERENTIAL SELECTIVITY OF DEGENERATION WITHIN CA1 REGION OF THE RAT HIPPOCAMPUS. S. Mennerick, D. Lutz, R.L. Dean, and R.T. Bartus, CORTEX Pharmaceuticals, Inc., Irvine, CA 92718

Kainic acid (KA) and trimethyltin (TMT) are two neurotoxins that apparently work through different mechanisms, yet share the feature of being relatively selective at low doses for hippocampal CA3 neurons. At higher doses both toxins recruit other hippocampal neurons as well as cells in other limbic associated structures. However, we report here that in the rat, using a silver stain (selectively staining degenerating neurons), at doses high enough and at time points long enough to demonstrate damage in the hippocampal CA1 subfield, a subtle difference emerges in the neuropathology induced by each toxin. While administration of KA (ICV and intrahippocampal infusions) at moderate doses recruits neurons clearly localized to the stratum pyramidale of CA1, systemically administered TMT effects are localized to cells bordering on CA1 stratum oriens. At higher doses this selective effect is lost and cells within stratum pyramidale are recruited by TMT and cells in the oriens are recruited by KA.

While the KA effect on CA1 pyramidal cells has been discussed at length previously, this is the first discussion of the preferential vulnerability to TMT toxicity of cells residing on the oriens border of the CA1 pyramidal cell layer. Rostral to caudal and medial to lateral gradients of the selectivity of the TMT effect in CA1 are discussed as well as morphological aspects of argyrophilic cells in CA1. The differences in selectivity between the two toxins are discussed with respect to possible mechanisms of TMT and KA toxicity toward hippocampal CA1 neurons.

454.10

IS TAURINE INVOLVED IN THE PATHOGENESIS OF HEPATIC ENCEPHALOPATHY? S.S. Oja, P. Saransaari and U. Wysmyk*. Tampere Brain Research Center, Department of Biomedical Sciences, University of Tampere, Box 607, SF-33101 Tampere, Finland, and Medical Research Centre, Polish Academy of Sciences, 00-784 Warsaw, Poland.

The possible role of taurine in hepatic encephalopathy (HE) was studied with rats injected with thioacetamide (TAA). The spontaneous release of exogenous labeled taurine from superfused tissue slices was not affected in any brain area studied but the potassium-stimulated release was significantly enhanced in the striatum. An exposure of striatal slices to ammonium ions in vitro induced concentration-dependently taurine release. In this respect ammonium ions were more effective than potassium ions. The ammonium ion stimulation was also attenuated in the presence of depolarizing concentrations of potassium ions. In striatal slices the ammonium-stimulated release of taurine was more pronounced in TAA-treated than control animals. The greater potassium and ammonium stimulation of taurine release from striatal slices in TAA-treated rats suggests that taurine may participate in the pathogenesis of HE. Supported by the Polish Academy of Sciences and the Emil Aaltonen Foundation, Finland.

455.1

NEUROTOXIC EFFECTS OF CYCASIN ON MURINE CORTICAL ORGANOTYPIC CULTURES. G.E. Kisby, B.G. Gold, and P.S. Spencer. Center for Research on Occupational and Environmental Toxicology, Oregon Health Sci. Univ., Portland, OR 97201.

Cycasin and its aglycone methylazoxymethanol (MAM) are under study as etiological candidates of a prototypical human neurodegenerative disorder with features of amyotrophic lateral sclerosis, Parkinsonism and Alzheimer's disease. Cycasin was isolated from frozen *Cycas circinalis*, L. seed kernels (Guam) and purity established using UV, TLC and HPLC. Murine cortical explant cultures were grown *in vitro* for 2 weeks and treated every other day for 5 days with 50 µM sucrose (control; minor contaminant of crude cycasin) or 10 µM crude cycasin. A majority of cycasin (83 %) and low levels of MAM (<1%) were detected by HPLC in media from cultures treated for 5 days. Thick (1 µm) and thin sections of explants were examined by light and electron microscopy at 1-5 days.

At 1 day, some cell bodies demonstrated vacuolated cytoplasm by light microscopy. By day 3, neuronal cell body necrosis was prominent and extensive vacuolation was observed in the neuropil. Vacules were markedly increased in size at 5 days. Ultrastructrually, these vacuoles consisted of swollen dendritic processes, sometimes associated with recognizable synaptic terminals. Large vacuoles appeared to form from fusion of neighboring swollen processes. Nerve cell bodies also showed marked abnormalities, including patches of dense nuclear chromatin, aggregated ribosomes, swollen mitochondria, and a vacuolated cytoplasm. Stacks of swirled membranes, which appeared to represent disorganized rough endoplasmic reticulum, were found in some neuronal cell bodies.

These observations provide direct evidence that crude cycasin, either directly or via a metabolite, has neurotoxic potential. Based upon the pattern of neuronal damage, the responsible agent might elicit neuronal damage via an excitotoxic mechanism. [Supported by NIH 19611]

455.2

THE NEUROTOXIC EFFECT OF BMAA ON MOTOR NEURONS MAY BE MEDIATED BY ANDROGEN-DEPENDENT CELLULAR FUNCTION. R.C.Yu, R.Hiipakka* and B.G.W.Arnason. Dept. of Neurology and Ben May Lab., University of Chicago, Chicago, Il 60637.

The high incidence of amyotrophic lateral sclerosis(ALS) among the indigenous people of the Marianas Islands, whose diet contained cycads, is linked to the neurotoxic amino acid β-N-methylamino-alanine(L-BMAA), isolated from cycads seeds. Synthetic BMAA induces neuronal injury in culture and causes cynomolgus monkeys to develop neuro-pathological syndromes that closely resemble ALS(extrapyramidal disorders). The molecular mechanism underlying the specific vulnerability of motor neurons(MN) to BMAA remains unknown. MN contain a high level of androgen receptors(AR) and the topographic distribution of AR in the cranial MN of rats correlates with susceptability to the disease. To examine the possible role of AR in the etiology of ALS we studied the effect of BMAA on AR. Rat prostate tissue or a human prostate adenocarcinoma cell line (LNCaP) were exposed to 10^{-4} to 10^{-6} M BMAA for 0.5 to 18 hr. L-β-Oxalyl-α,β-diaminopropionic acid (L-BOAA), (a isomer derived from the Lathyrus plant known to cause cortical disorders) was used as control. We find that BMAA, but not BOAA, interferes with androgen-dependent cell function and its effect is dose-dependent. A 30 min exposure to 10^{-4} M BMAA reduces AR levels in the nuclear fraction of rat prostate tissue by 23%. An 18 hr. exposure to 3 mM BMAA reduces the amount of AR by more than 20% in both nuclear and cytosol fractions of LNCaP cells. This notion of a role for AR in the etiology of ALS is further strengthened by our earlier findings that cyproterone (an androgen antagonist) can significantly reduce the choline acetyltransferase level in spinal cord. Our results suggest a mechanism whereby a common factor can kill the MN in certain regions but spare others within the same CNS; similar mechanisms may be operative in other neuro-degenerative diseases.

455.3

PROCESSED CYCAD FLOUR EXTRACT NEUROTOXICITY IN NEURONAL CELL CULTURE. M.W. Duncan, A.M. Marini, S.P. Markey†* and I.J. Kopin. CNB, NINDS, NIH, & †SAB, NIMH, Bethesda, MD 20814.

A link between cycads and a variant of amyotrophic lateral sclerosis (ALS) in the western Pacific has been suggested. Therefore, we have tested extracts of female gametophyte tissue from *C. circinalis, C. revoluta* and *C. media*, as well as cycad flour, for neurotoxicity using cultured cerebellar granule cells and cultured mesencephalic cells. Neither seed extracts nor 13 of the 17 processed flour samples were significantly more toxic than wheat flour. However, 4 of 17 processed cycad flour extracts exhibited marked neurotoxicity. Analysis of these extracts for the neurotoxin 2-amino-3-(methylamino)-L-propanoic acid (BMAA) indicated that there was no correlation between toxicity and BMAA content, and the BMAA concentrations in the medium were far below those required to kill cultured neurons. In addition, MK-801 did not protect against the neurotoxicity, indicating this response was not mediated via the N-methyl-D-aspartate (NMDA) receptor. The toxic principle could not be extracted into an organic solvent at acidic, basic or neutral pH and was heat and acid stable. All 4 toxic samples were subsequently found to have high content of zinc and this metal was shown to be responsible for the neurotoxicity. We conclude that cycad extracts are not significantly more toxic to cultured neurons than is wheat flour, and that the marked neurotoxicity of 4/17 processed cycad flour samples derived from *C. circinalis* was mediated via zinc. These findings may link zinc to ALS on Guam, perhaps by exacerbating a long-term imbalance in essential trace minerals, or by the direct neurotoxic actions of zinc itself.

455.4

ESTROGEN-LIKE EFFECTS OF THE ORGANOCHLORINE INSECTICIDE CHLORDECONE ON LORDOSIS BEHAVIOR. H. Brown* and L. Uphouse. Dept. of Biology, Texas Woman's Univ., Denton, TX, 76204.

The organochlorine pesticide chlordecone acts similarly to estrogens at the chick oviduct: chlordecone binds to oviductal estrogen receptors, induces mRNAs for the proteins ovalbumin and conalbumin, induces the proteins themselves and their secretory apparatus, and initiates oviductal growth. In contrast, estrogen-like effects of chlordecone in neural tissues are understood less thoroughly. For example, chlordecone treatment (50 mg/kg ip), when combined with treatment with 0.5 mg progesterone (P) sc, failed to substitute for estradiol in priming for the lordosis response (Uphouse, L., et al., NeuroToxicology 7: 127, 1986), a behavior known to require estrogen action in the brain. Recently, we reassessed chlordecone's capacity to prime for the lordosis reflex in response to male mounting behavior by giving ovariectomized rats a single injection of 50 mg/kg chlordecone ip followed 44 or 67 hr later by injection of 2 mg P im. When observed 5 to 6 h or 9 to 13 h after P treatment, these rats showed mean ± SE lordosis quotients of 57.9 ± 11.9 and 88.5 ± 5.1. The priming effect of chlordecone was antagonized markedly by injections of 2 mg nafoxidine ip at -24 h and at +24 h relative to the chlordecone injection. Chlordecone bound (K_i = 8 x 10^{-8} M) to cytosolic estrogen receptors from neural areas implicated in the control of lordosis behavior; the collective evidence indicates that chlordecone substitutes for estrogen in priming for the lordosis response of ovariectomized rats, and that the pesticides's effects on lordosis behavior are mediated by neural estrogen receptors.

455.5

THE MECHANISM AND DYNAMIC PATTERN OF SOMAN INDUCED BRAIN HISTOPATHOLOGY - A MORPHOMETRIC STUDY. S. Shapira and T. Kadar*, Dep. Pharmacology, IIBR, Ness-Ziona 70450, ISRAEL.

Soman, a highly toxic cholinesterase (ChE) inhibitor, causes chronic brain lesions through a yet unknown mechanism. The present study was designed to clarify the mechanism leading to soman induced brain lesions by comparing them to the effect of either a non-ChE inhibitor convulsant (metrazol) or another toxic ChE inhibitor (DFP) in equipotent (1LD50) doses. Following administration, surviving rats were sacrificed at various time intervals and their brains evaluated by histological and morphometric analysis. All three substances produced severe toxic signs, including convulsions. Soman and DFP also generated signs of cholinergic hyperactivity. Soman injected rats developed CNS lesions already 24 hr after administration, mainly in the hippocampus, frontal and pyriform cortex and thalamus. In three months these lesions have spread to areas which were not involved initially. Morphometric analysis revealed the dynamic pattern of the lesions. No morphological changes were noted in any of the metrazol or DFP injected rats. These findings strongly suggest that soman induced CNS lesions are caused by a direct effect of soman on specific brain areas, and not by convulsions per-se or by ChE inhibition per-se.

455.6

EFFECTS OF THREE REPUTED CARBOXYLESTERASE INHIBITORS UPON RAT SERUM ESTERASE ACTIVITY J.P. Chambers[1], S.L. Hartgraves[2], M.R. Murphy[2] and J.J. Valdes[3]. [1]The University of Texas at San Antonio, San Antonio, TX 78285, [2]Radiation Sciences Division, USAF School of Aerospace Medicine, Brooks AFB, TX 78235 and [3]U.S.Army Chemical Research Development and Engineering Center, Biotechnology Division, Aberdeen Proving Ground, MD 21010.

Rats have very high endogenous levels of serum carboxylesterase (CAE) which accounts for the lower sensitivity of rats to toxic organophosphates. In this study, the effects of three reputed CAE inhibitors, 2-(o-Cresyl)-4H-1:3:2-benzodioxaphosphorin-2-oxide (CBDP), bis-p-nitrophenyl-phosphate (BNPP), and tetraisopropyl pyrophosphoramide (Iso-OMPA) on the hydrolysis of several substrates were determined. Respective kinetic constants 'K_m apparent and V_{max}' were derived and inhibitory effects compared using saturating amounts of substrate. Using slab polyacrylamide gel electrophoresis and densitometric scanning, the effects of these inhibitors upon hydrolysis of various substrates by rat serum esterases were monitored and quantitated.
Research supported by AFOSR.

455.7

THE USE OF CARBOXYLESTERASE INHIBITORS TO DEVELOP AN IMPROVED RODENT MODEL OF SOMAN TOXICITY. M.R. Murphy, S.Z. Kerenyi, S.A. Miller, J.P. Chambers, R.F. Noonan[1], and S.L. Hartgraves. USAF School of Aerospace Med., and [1]Systems Research Laboratories, Brooks AFB, TX 78235.

Carboxylesterases (CaE) (aka aliesterases) are non-specific B-esterases of unknown function, except possibly the detoxification of xenobiotics. Because of the rat's high natural level of serum CaE, this species is much less sensitive, relative to primates, to highly toxic organophosphate anticholinesterases such as soman. The purpose of our work was to develop the CaE-inhibited rat as an improved (i.e., more primate like) model of soman toxicity.

We report the results of studies on (1) a comparison of the CaE inhibitors (CaEIs): CBDP, iso-OMPA, and BNPP; (2) the time course of CaE recovery following CaEI administration; (3) the effects of pyridostigmine, physostigmine, and soman on CaE levels; (4) the effects of CaEIs on: brain cholinesterase (ChE), serum ChE, and spontaneous activity; (5) the 7-day chronic inhibition of CaE; and (6) the toxicity of CaEIs.

Research was supported by the USNMRDC.

455.8

SOMAN TOXIC SYNDROME (STS). S.A. Miller[1*], M.R. Murphy[1], S.Z. Kerenyi[1*], D.L. Armstrong[3], R.C. Switzer III[2], D.W. Blick[2], S.L. Hartgraves[1]. [1]Radiat. Sci. Div., USAF Sch. Aerosp. Med., and [2]Systems Research Laboratories, Brooks AFB, TX 78235, [3]UTSA.

STS is a soman-induced disorder characterized by extensive, but localized, brain damage and hyperreactivity. In this study, we further examine the nature of this syndrome. In addition to a normal control group (CON), a weight loss control (WLC) group was included to approximate the severe weight loss and subsequent recovery of the STS group. Animals developing STS received extra care (e.g., special diet, dextrose injections, and bedding material). WLC animals were treated similarly.

STS animals displayed hyperreactivity to stimuli in a two-way shuttlebox, but learning was unaffected. However, STS animals did exhibit a loss of long term potentiation in the in vivo hippocampus. In automated activity monitors, STS rats habituated rapidly to the novel environment and exhibited less total exploratory behavior. When given 1.5 mg/kg d-amphetamine, STS animals showed a much more dramatic increase in activity than did WLC animals (Hartgraves *et al.*, these proceedings).

455.9

EFFECTS OF STIMULANTS ON LOCOMOTOR ACTIVITY IN RATS WITH SOMAN TOXIC SYNDROME (STS). S. L. Hartgraves, M. S. Tristan*, S. Z. Kerenyi*, S. A. Miller*, and M. R. Murphy. USAF School of Aerospace Medicine, Brooks AFB, Texas, 78235-5301; University of Texas Health Sciences Center, San Antonio, Texas, 78284.

Soman Toxic Syndrome (STS) is a soman-induced, seizure-related syndrome characterized by extensive, but localized, brain damage, hyperreactivity to physical stimuli, and general hypoactivity (see abstract by S. A. Miller, et al., this conference). The objective of this research is to further analyze the locomotor behavior (measured in automated activity monitors) of STS animals by administering stimulant drugs.

STS animals respond to d-Amph (1.5 mg/kg, s.c.) with 3-fold increases in distance traveled and rearing compared to weight loss controls (WLC). STS animals also show over 20-fold increases in these same parameters compared to their responses to saline injections, whereas WLC animals show only 3-fold increases in distance traveled and rearing (d-Amph vs saline, crossover).

We report here a comparison of STS dose-response curves for d-Amph and for caffeine, a stimulant that enhances locomotor activity independently of the nucleus accumbens (which is closely associated with d-Amph-induced locomotor activity).

455.10

REGENERATION OF ACETYLCHOLINESTERASE IN THE CLONAL NEUROBLASTOMA-GLIOMA HYBRID NG108-15 CELL LINE AFTER SOMAN INHIBITION: EFFECT OF GLYCYL-L-GLUTAMINE. J.J. Yourick, P.A. Eklo, M.P. McCluskey and R. Ray. US Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, MD. 21010-5425.

Acetylcholinesterase (AChE) in the clonal NG108-15 cell line has been previously characterized. This cell line may represent a model system in which to study AChE regulation and effects of chemical compounds that may alter AChE activity (Ray et al., Soc. Neurosci. Abstr., 12:889, 1986). Recently, it was shown that glycyl-L-glutamine (GLG) may function as a neurotrophic factor for maintenance of AChE content in denervated superior cervical ganglion of the cat (Koelle et al., 1988). Studies were conducted in undifferentiated NG108-15 cells to characterize the regeneration (de novo synthesis) of AChE activity after soman inhibition. In addition, the effect of GLG on AChE regeneration after soman treatment was investigated. Cells were grown to confluence, treated with soman (10^{-6} M) for 15 min and washed to remove excess soman. GLG (10^{-6}, 10^{-5} or 10^{-4} M), glycyl-L-glutamic acid (10^{-6} M) or vehicle was added to the culture medium immediately after soman treatment and remained in the medium until cell harvest. Cells were harvested at 1, 3, 5, 12, 20, and 24 hours after soman treatment and specific AChE activity was determined. After soman, AChE activity dramatically decreased to less than 1% of control activity at 1 hr. The regeneration of AChE activity gradually increased at 5 (7%), 12 (14%) and 20 (85%) hours, while complete AChE activity was regained 24 hr after soman. GLG and glycyl-L-glutamic acid, at all concentrations tested, did not increase the rate of AChE de novo synthesis after soman inhibition. These studies have characterized AChE regeneration after soman inhibition in the NG108-15 cell line for potential use as a model system to study compounds that alter AChE activity. In this model system, GLG and glycyl-L-glutamic acid failed to act as a neurotrophic factor for AChE synthesis.

455.11

HYPERAMMONEMIA, IN THE ABSENCE OF GLUTAMINE SYNTHESIS, DOES NOT ALTER BRAIN FUNCTION. J.Jessy*, M.R.DeJoseph* & R.A.Hawkins. Dept. of Physiology and Biophysics, University of Health Sciences / The Chicago Medical School, North Chicago, IL 60064.

Portacaval shunted rats show many of the typical metabolic alterations found in humans with hepatic encephalopathy. Most of the characteristic changes are established within 24-48 h after surgery and include: raised plasma and brain ammonia, depressed brain function and increased transport of neutral amino acids across the blood-brain barrier. Ammonia is suspected to be an important etiologic factor. An earlier study from this laboratory showed that hyperammonemia caused most of the changes found after shunting, and that the depression of cerebral function was more closely correlated to glutamine, a metabolite of ammonia, rather than ammonia itself. The present experiments were designed to address the question whether ammonia alone, in the absence of net glutamine synthesis in brain, could be responsible for cerebral dysfunction. Plasma ammonia levels in normal rats were raised to values similar to those found after portacaval shunting by the administration of small doses of methionine sulfoximine, an inhibitor of glutamine synthetase. Cerebral energy metabolism in methionine sulfoximine-treated rats remained unaltered. There was no depression in brain glucose consumption. Key intermediary metabolites and high energy phosphates were normal and neutral amino acid transport (tryptophan and leucine) was unchanged. These results indicate that hyperammonemia, when not accompanied by net glutamine synthesis in the astrocytes, does not result in cerebral dysfunction. (Supported by NS16389 and NS 16737).

455.12

PURIFICATION OF ARYLAMINE N-ACETYLTRANSFERASE FROM RAT BRAIN. S.Gaudet, M.Palkovits*, and M.A.A.Namboodiri, Dept. of Biol. Georgetown University, Washington, D.C. 20057,*Lab. Cell Biol.NIMH, NIH, Bethesda, Md. 20892.

The brain arylamine N-acetyltransferase (NAT) activity is about 10-fold higher than the arylalkylamine NAT present in the same tissue. The function and regulation of the arylamine NAT is not known at this time. Recent molecular cloning studies indicate that multiple arylamine NATs, based on their substrate specificity, may exist in different tissues. In the present study, we have investigated the above possibility with respect to rat brain.

Arylamine NAT activity was assayed in micropunch samples, using p-phenetidine as the amine substrate, and was found to be evenly distributed in different areas of the brain. The enzyme activity was purified via a three step procedure involving $(NH_4)_2SO_4$ precipitation, affinity chromatography using methotrexate, and finally, size exclusion HPLC. Analysis of the final preparation using SDS-PAGE showed one major band (Mr=30K) and a few minor bands. The Mr of the native enzyme was found to be approximately 31K. Substrate specificity studies indicate that the enzyme is an arylamine NAT, with only 1-2% activity towards arylalkylamines (i.e. tryptamine). The remarkably high specificity of this enzyme towards arylamines indicates that the brain enzyme may differ from that found in liver. (Supported by NIH grant, DK 37024 to MAAN).

455.13

INCREASED RESISTANCE TO CHRONIC ORAL 3-NITROPROPIONIC ACID [3NPA] NEUROTOXICITY IN AGED MICE: EM AND HISTOCHEMICAL STUDIES. Y.D. Tan*, T. Bohlmann*, G.S. Nizamuddin*, F.S. Chu*, R.L. Sufit, B.R. Brooks and H.S. Schutta Neuro. Svc., Wm. S. Middleton VA Hosp. and Neuro. Dept., Univ. Wisconsin Sch. of Med., Madison, WI 53792

Chronic ingestion of the nitroaliphatic neurotoxin 3NPA, a suicide inhibitor of SDH, results in intraneuronal glycogen accumulation in the spinal cord in young mice. The acute single dose LD_{50} was identical in young [221 mg/kg; 95% confidence limits: 166-295] and old [205 mg/kg; 169-249] mice. Following daily gavage feedings, the chronic LD_{50} was significantly [$p<0.04$] higher in old [138 mg/kg; 133-144] compared with young [49 mg/kg; 47-51] mice. Succinic dehydrogenase [SDH] activity was decreased at 10 min but was absent at 30 min post 3NPA. Spinal cord intraneuronal glycogen accumulation was not seen in mice acutely fed 2.5 LD_{50} but was present following chronic feeding of 0.2 or 0.4 LD_{50} 3NPA. Aged mice demonstrated no increased resistance to acute toxic effects of 3NPA on spinal cord neurons but could withstand almost a 3-fold increase in the dose of chronically administered 3NPA. [Supported by MDA Midwest Regional ALS Research and Treatment Program grant and an MDA Research Fellowship grant].

455.14

SEX-RELATED DIFFERENCES IN TRIMETHYLOLPROPANE PHOSPHATE NEUROTOXICITY AND PHARMACOKINETICS. J. Rossi III, V.J. Forrest* and J. Gearhart*. Naval Medical Research Institute, Toxicology Detachment, Wright-Patterson Air Force Base, Ohio 45433 and NSI Technology Services Corporation, Dayton, Ohio 45431.

Thermal decomposition of trimethylolpropane-based compounds which contain fire retardant triarylphophate additives allows formation of neurotoxic trimethylolpropane phosphate (TMP-P), a caged convulsant believed to act via GABAergic receptor antagonism.

The effect of sex-related differences after dermally applied TMP-P was evaluated in rats (Fisher 344) with hormonally-altered sex phenotypes and in castrated adult rats with and without hormonal supplementation. Auditory startle was found to be greater in males than females after a sublethal dose of TMP-P (30 mg/kg). Additionally, males exhibited shorter times to convulsion and death than females after a lethal dose (120 mg/kg). Female rats dermally exposed to TMP-P exhibited greater tissue/blood partition coefficients than males. Phenotypically and surgically unaltered animals exposed to TMP-P or the solvent vehicle served as the positive and negative controls, respectively.

A physiologically based pharmacokinetic (PBPK) model was developed to describe the kinetics of TMP-P. The steady state partition coefficients of TMP-P in tissues and urinary excretion rate, determined in subcutaneously dosed animals, was used in the PBPK model to estimate the in vivo metabolism of TMP-P and to simulate the blood and tissue kinetics. The PBPK model was then used to derive a permeability rate constant for TMP-P transfer across rat skin and to explain the effects of tissue/blood partitions on brain levels of TMP-P.

455.15

NA-K ATPASE DECREASES IN THE BRAIN IN DIABETES. S.F.Leong. Physiology Dept., Natl. Univ. of Singapore, Kent Ridge, Singapore 0511.

The use of streptozotocin in animal models has shown to develop abnormalities in the nerve similar to those observed in diabetes. Reduced Na-K ATPase activity is often reported for the peripheral nervous system. This study establishes the Na-K ATPase in the regions of the brain during diabetes. Streptozotocin (60mg/kg body wt) was injected i.p. into male Wistar rats (250-300g) and housed for 7 days ad libitum. All animals were checked for diabetes before use. Reduced Na-K ATPase was observed in brain from diabetic animals. All regions showed significant reduction ranging from 31% in the hippocampus to 6% in the striatum and the brain stem. In contrast, a neurotransmitter marker enzyme, acetylcholinesterase, showed that most regions did not show significant changes in the diabetic brain for acetylcholine metabolism. These results showed that diabetes which produces hyperglycaemia not only commonly present reduced Na-K ATPase activity in the peripheral nervous system but also in the central nervous system. Such reduction in the brain, however, are not known widely to show degenerative effects seen in the peripheral nervous system.
(Supported by NUS Grant RP116/85).

455.16

NEUROTOXICITY FROM DIRECT CURRENT STIMULATION. R.J. Hurlbert, E. Theriault, C.H. Tator. Playfair Neuroscience Unit, University of Toronto, 399 Bathurst St., Toronto, CANADA M5T 2S8

The effect of continuous direct current stimulation on the normal adult rat spinal cord was assessed over a variety of intensities in 32 animals. Platinum/iridium stimulating electrodes of 2 sq.mm. surface area were implanted 10 mm. apart in an epidural fashion at T1/2, with battery packs buried subcutaneously at the incision. Stimulator intensities ranged from 0 to 18 uA. The animals were allowed to survive for a period of 2-12 weeks undergoing twice weekly inclined plane testing. Following this the spinal cords were harvested, sectioned at 8 um, and stained with Luxol Fast Blue and Hematoxylin and Eosin. Grossly, pathological changes such as discoloration and cavitation were seen under the anode after 3 weeks, with intensities as low as 3 uA. Inclined plane testing was sensitive only to the 18 uA induced changes. These results demonstrate the low tolerance of mammalian CNS tissue to DC stimulation and have implications for continued studies into its therapeutic usefulness.

455.17

NEUTRON/GAMMA IRRADIATION PRODUCES DIFFERENTIAL LOCOMOTOR DECREMENTS IN ISOLATED AND GROUP HOUSED MICE. H.D. Davis, M. Miernicki, M.E. Faccioli, and M.R. Landauer. Behavioral Sciences Department, Armed Forces Radiobiology Research Institute, Bethesda, MD 20814-5145.

Male CD2F1 mice that were either isolated for 5 days or group housed (10/cage) were exposed to 5 Gy of radiation at 1 Gy/min (neutron:gamma = 1:1), or sham irradiated (control) (N=16/group). Locomotor activity (ambulation) of individual mice was monitored for 12 hr postirradiation (PR). There was no significant difference in activity between the isolated and the group housed controls. Irradiated mice exhibited a locomotor decrement within 1 hr PR that fell to 32% of control levels by 4 hr PR in the group housed animals and 62% of control levels by 3 hr PR in the isolated animals. The isolated irradiated animals were not significantly different from the isolated control animals by 4 hr PR, while the group housed irradiated animals remained significantly different from their controls until 8 hr PR. Thus, radiation-induced locomotor decrements in isolated animals were both less pronounced and of shorter duration than those that appeared in group housed animals. This effect cannot be due simply to increased overall activity produced by isolation, because the isolated and group housed controls exhibited similar levels of activity. These findings represent differential effects of radiation in animals housed under different social conditions, with social isolation attenuating the behavioral effects of radiation.

455.18

MODIFICATION OF ACOUSTIC STARTLE REFLEX FOLLOWING EXPOSURE TO IONIZING RADIATION. D.E. Morse and K.M. Manderfield*. Behav. Sci., AFRRI, Bethesda, MD 20814

Inhibition of the acoustic startle reflex, by the presentation of a pre-startle pulse stimulus, has been shown to be sensitive to changes in sensory and motor capacity following toxin exposure. It has been reported that exposure to ionizing radiation, at sublethal doses, severely depresses locomotion and appetitive behavior. The data suggest that these behavioral effects are related to changes in endogenous opioid and dopamine activity. It is not clear whether changes in locomotor capacity or sensory function contribute to these effects. In the present study, male Sprague Dawley rats (300-425g) were exposed to ionizing radiation (1-10 Gy, Linac 18 MVp electrons, 10 Gy/min), or were administered naltrexone (.01-5 mg/kg). The amplitude of the startle response (Startle pulse: 122 dB, 25 ms dur., 2 ms rise), on trials where a pre-stimulus preceded (100 ms) the startle pulse, was compared to startle amplitude when the startle stimulus was presented alone. Baseline responding was measured in a separate session. The results suggest that neither radiation exposure or opioid blockade reduce the amplitude of the response to the startle-eliciting stimulus (when presented alone). However, prepulse inhibition of the startle reflex was significantly reduced following radiation exposure or Naltrexone administration. These data suggest that postirradiation behavioral suppression is not caused by decreased motor capacity, but instead may be mediated by changes in sensory function.

455.19

SEIZURES AND NEUROGENICALLY-MEDIATED CARDIAC ARRHYTHMIAS INDUCED BY BUPIVACAINE IN RATS. F.G. Zavisca,* J.E. Heavner, J. Kytta*, M. Badgwell.* Anesthesia Dept., Texas Tech Univ., HSC, Lubbock, TX 79430.

Bupivacaine is a local anesthetic that produces seizures and cardiac arrhythmias, the latter possibly by a neurogenic mechanism. We examined the relative CNS and CV effects of bupivacaine in five lightly-anesthetized male Wistar rats (264-324 g). EKG and EEG leads, and femoral artery and vein cannulas, were placed under halothane anesthesia. Muscle paralysis was induced with pancuronium 0.1 mg/kg IV and respiration controlled. Body temperature and arterial blood gases were maintained in a narrow range near normal values for the rat. Then, light anesthesia was maintained with 0.5% halothane, 70% N_2O and 30% O_2 for 30 minutes. Bupivacaine infusion 2 mg/kg/min IV was started. Four endpoints were noted in all rats. These endpoints and dose required (mg/kg, mean ± SE) were: 1) dysrhythmia 4.22 + .84; 2) seizures 7.08 + .69; 3 isoelectric EEG 11.05 ± 2.35; and 4) asystole 20.40 ± 2.90. Investigations using this preparation may yield significant new information regarding seizure mechanisms and neurogenic influences on cardiac rhythm.

455.20

OXIDATIVE STRESS ENHANCES PYRUVATE AND PALMITATE OXIDATION IN PC12 CELLS. M. M. Haileck* and F. C. Kauffman. Lab. for Cell. and Biochem.Toxicol., Rutgers University, Piscataway, NJ 08854. Maintenance of high energy phosphates (~P) is compromised in various cells during oxidative stress. Rat pheochromocytoma cells (PC12) exposed to H_2O_2 provide a highly reproducible model to study mechanisms of oxidant injury (Toxicologist **10**:520, 1990) . Cells injured reversibly with 0.1 mM H_2O_2 show transient decreases in ~P whereas cells injured irreversibly (0.25 mM H_2O_2) fail to recover ~P and this event precedes cell death as indexed by loss of adenylate kinase. Energy failure likely occurs subsequent to inhibition of glycolysis and/or oxidative metabolism by H_2O_2 . Accordingly, we examined the oxidation of [6-^{14}C] glucose, [1-^{14}C] pyruvate and [1-^{14}C] palmitate in cells pretreated with 0.1 mM H_2O_2 or 0.25 mM H_2O_2 . Substrates were added 10 min after addition of H_2O_2 to cells. Under experimental conditions employed, H_2O_2 was degraded completely by cells ($t_{1/2} \approx 1$ min).

	Oxidation of ^{14}C-Labelled Substrates		
	[6-^{14}C] Glucose*	[1-^{14}C] Pyruvate **	[1-^{14}C] Palmitate*
Control	25.7	3.59	5.28
0.1 mM H_2O_2	18.9	5.77	9.01
0.25 mM H_2O_2	46.9	6.47	14.88

* pmole/min/mg protein ** nmole/min/mg protein

The high yield of $^{14}CO_2$ from pyruvate compared to that obtained with glucose suggests that mitochondrial oxidation of pyruvate is limited by substrate availability. Cells injured irreversibly with 0.25 mM H_2O_2 oxidized [6-^{14}C] glucose at higher rates than control suggesting that glycolysis was not impaired. Pyruvate and palmitate oxidation was stimulated by H_2O_2 indicating that the capacity of mitochondria to oxidize these substrates was also not compromised by the oxidant. Higher rates of oxidation of mitochondrial substrates in the presence of reduced ~P argues strongly that mitochondria are uncoupled in PC12 cells subjected to irreversible oxidative stress. (Supported in part by ES-05022)

455.21

NEUROPATHOLOGY IN THE RETROSPLENIAL CORTEX (RSPC) OF RATS AFTER DEXTROMETHORPHAN (DM) ADMINISTRATION. M.P. Weisend, D. Heard* and D.M. Feeney. Depts.of Psych. and Physiol., Univ. of New Mexico, Albuq., NM 87131.

We previously reported that DM (75 or 50mg/kg;ip) evokes convulsions accompanied by EEG seizure activity in normal male rats (Heard et.al.,Soc.Neurosci.Abst.15:1214, 1989). Because DM is a noncompetitive N-methyl-d-aspartate antagonist and other such drugs produce RSPC pathology, we conducted light microscopic analyses of thionin, thionin/acid fuchsin-stained sections and cytochrome oxidase (CYO) histochemistry for neurotoxic effects of DM. Male rats were randomly assigned to receive 35mg/kg, 50mg/kg or saline every 48hr (6-8 injections) or 75mg/kg once every 24 or 48hr (2-5 injections). Histology was independently rated (interrater reliability r=.8698) for RSPC pathology by observers uninformed of treatment. Pathology characterized by swollen neuronal somata with poorly stained cytoplasm was seen in the pyramidal and deep granule cell layers of the posterior RSPC. Thionin-stained sections indicated statistically significant increases in RSPC pathology with increasing doses of DM. Data from CYO and acid fuchsin is to date inconclusive. There was considerable variability amongst animals, perhaps due to duration of fixation, deaths at high doses, and/or seizure frequency. Supported by DHHS Grants R01NS20220-03, 3-S06-RR08139 and a Biomedical Research Support Grant from the University of New Mexico.

NEUROTOXICITY: OTHER II

456.1

MORPHOMETRIC NEUROHISTOLOGICAL STUDIES OF RHESUS MONKEYS AFTER CHRONIC MARIJUANA SMOKE (MS) EXPOSURE. A.C. Scallet, E. Uemura, A. Andrews*, J. Craven*, R. Rountree*, S. Wilson*, S.F. Ali, J.R. Bailey*, M.G. Paule and W. Slikker, Jr*. Div. of Reprod. & Develop. Tox. National Center for Toxicological Research, Jefferson, AR 72079.

Monkeys were exposed daily for one year to MS. High-dose monkeys smoked a single cigarette (2.6% THC) 7days/wk, while low-dose animals smoked only 2days/wk. Control groups were either sham-exposed or smoked ethanol-extracted marijuana 7days/wk. Seven months after the final dose, the monkeys' hippocampi (HIPP) were evaluated by quantitative dendritic analysis (Golgi-method) or by electron microscopy. Results revealed no effects of MS on the total volume of monkey HIPP, nor on the volumes of its major subdivisions. There were also no net effects of MS on synaptic characteristics, neuronal size, the number of apical or basilar dendrites or the number or length of dendritic branches. However, sham-handled monkeys that were performing operant tasks daily throughout the year of treatment had more highly branched (+ 55%, $p < 0.05$) and longer (+ 47%, $p < 0.01$) dendrites and had fewer mossy-fiber synapses per unit volume (-21%, $p<0.05$) than monkeys that did not perform these tasks throughout the year. Our results indicate that cognitive variables are of importance in neurohistological studies of psychoactive drugs, but fail to confirm previous findings of neurohistological alterations in THC-treated rats. (Supported by NIDA IAG #224-83-005 and NCTR E-6230).

456.2

LEAD INHIBITS PURIFIED PROTEIN KINASE C SUBTYPES FROM RAT BRAIN. G.P. Feng, S.G. Chen, K. Murakami. Dept. of Biochem. Pharmacol., State Univ. of New York, Buffalo, NY 14260.

Protein kinase C (PKC) is an important enzyme in mediating cellular signal transduction. Recently it has been reported that picomolar concentrations of lead activate partially purified PKC from rat brain (Nature 334, 71-73, 1988). Since lead is a neurotoxic heavy metal and its toxicity may be related to the alteration of PKC activity in vivo, we examined lead effects on PKC activation. PKC from brains of Sprague-Dawley rats was purified using a four step liquid chromatography procedure. PKC subtypes I, II and III were further separated using a hydroxy-apatite FPLC column.

Our results showed that lead had strong inhibitory effects on PKC activity of these subtypes as follows:
(1) Submicromolar concentrations of lead acetate strongly inhibited PKC activity induced by phosphotidylserine (PS) in the presence of calcium.
(2) Lead did not mimic the calcium effect on PKC activation in the presence of PS.
(3) Oleic acid activate PKC without calcium. This activation of type I and II PKC was also inhibited by lead acetate at the same concentrations.

Such strong inhibitory effects of lead on PKC may be related to its neurotoxicity, such as memory disorders.

456.3

COMPARISON OF METHODS FOR THE HIGH THROUGHPUT (96 WELL PLATE) DETERMINATION OF LDH RELEASE FOR ASSESSMENT OF NEUROTOXICITY IN LOW DENSITY NEURONAL CULTURES. D. L. Needels. CNS Biology, Bristol-Myers Squibb Co., Wallingford, CT 06492.

The release of cytoplasmic enzymes such as lactate dehydrogenase (LDH) has long been used as a marker for cellular damage, with at least 2 protocols adapted to measurement in 96 well plates. In the absence of serum (which contains LDH), it should be possible to assay LDH released by much lower density neuronal cultures than previously used.

Three assay systems were compared: 1) Forward Reaction (increased NADH, 340 nm); 2) Reverse Reaction (decreased NADH, 340 nm); and 3) Colorimetric Reaction (NADH coupled to tetrazolium salt, 490 nm). Each method was found to have its own advantages and disadvantages in terms of thermodynamics, linear range, sensitivity, and variability. Kinetic analysis eliminates two problems associated with a simple endpoint measurement; relative timing from one well to another, and variations in initial absorbance. Surprisingly, there was no apparent advantage to a linear least squares fit over simply using the first and last data points (double endpoint method). With relatively noisy data (*e.g.* at high OD), equivalent results could be obtained by averaging the first three and last three data points before calculating the slope. Although a quadratic fit modeled the overall reaction curve much better, the variability of initial rates was greater than that observed in the slope of linear fits.

Each of these methods can be used to assay LDH at the levels released by low density (≤ 10,000 neurons/cm2) cultures of hippocampal neurons in response to NMDA. The choice of method will depend on equipment (UV *vs.* visible optics), software (kinetic readings), the LDH range, the number of plates to be assayed, and data analysis capability.

456.4

CHOLINERGIC SEPTOHIPPOCAMPAL NEURONS UNDERGO MORPHOLOGICAL ABERRATIONS FOLLOWING LOCAL APPLICATION OF THE NEUROFILAMENT-DISRUPTING AGENT 2,5-HEXANEDIONE (HD). P. L. Di Patre and L. L. Butcher. Dept. of Psychology, UCLA, Los Angeles, CA 90024-1563, U.S.A.

We previously demonstrated that intrafimbrial (i.f.) injection of the microtubule-disrupting agent colchicine induces morphological alterations in fibers running through the distal fimbria and stained with acetylcholinesterase (AChE) and nerve growth factor receptor (NGFr) (Di Patre et al., *Brain Res.*, 1990, in press). In this investigation, we studied the morphological effects of i.f. injection of HD, a drug known to produce axonopathies characterized by local accumulations of neurofilaments. Adult Sprague-Dawley rats were used and i.f. stereotaxical injections were carried out as previously reported (above ref.). Two and 4 days after i.f. injections of HD 0.5 mg, we found that many AChE-NGFr positive axons in the most rostral part of the fimbria were much thicker and grossly distorted as compared to fibers in the homologous region of control rats. At 7 days post-treatment, the affected fibers became more distorted and showed occasional short collateral branches, which resembled newly sprouting ramifications. A reduced number of choline acetyltransferase-reactive neuronal bodies in the lesioned hemiseptum was also observed. A complete recovery of all these changes was seen 6 weeks after HD-treatment. Our data show that i.f. injections of cytoskeletal toxins may constitute a useful experimental model to study the role of the different cytoskeletal components in controlling axonal shape and in regulating the growth of fibers. [Support: NS 10928]

456.5

INCREASE IN CORTICAL BRAIN MICROVASCULARIZATION FOLLOWING CHRONIC ALCOHOLIZATION, CORTICAL INSULT AND AGING IN THE RAT. M. Gewiss*, Ch. Heidbreder* and Ph. De Witte. Lab. of Psychobiology, University of Louvain, Croix du Sud 1, B-1348 Louvain-la-Neuve, Belgium.

The alcoholization by an inhalation procedure seems to be one of the most appropriate experimental models to obtain and maintain chronically high blood ethanol levels. After sejourning 2 to 4 weeks into the alcoholization chamber, rats were perfused with a nuclear emulsion allowing to reveal the cortical vascularization. Furthermore, we also examined the issue of whether chronological ageing and cortical lesion were able to modify cortical microvasculature in non-alcoholized rats. All the vessels including terminal and lateral branches, were measured and the lengths were summed up. Our results provide evidence that animals chronically alcoholized from 2 to 4 weeks displayed enhanced cortical microvascular network. A similar enhancement of microvasculature was observed 1) in the cortex of aged animals (2 years old) when compared to normal adult rats and 2) at the edge around a lesion-induced cavity performed in the cortex of non-alcoholized rats by contrast to the contralateral intact side on the same section. These results show that chronic alcoholization, chronological ageing and brain injury share in common cortical hypervascularization.

456.6

MODULATION OF [^{35}S]t-BUTYLBICYCLOPHOSPHOROTHIONATE KINETICS BY DELTAMETHRIN IN RAINBOW TROUT BRAIN MEMBRANES. R.L. Golden and T.F. Murray. Toxicology Program and College of Pharmacy, Oregon State University, Corvallis, OR. 97331.

Pyrethroid insecticides are known to attenuate [^{35}S]t-butylbicyclophosphorothionate binding to gamma-aminobutyric acid (GABA) gated chloride channels in the vertebrate CNS. Deltamethrin, a Type II pyrethroid, has been shown to inhibit the equilibrium binding of [^{35}S]TBPS in rainbow trout membrane preparations (Eshleman and Murray, Neuropharmacol., in press). To investigate the mechanism of deltamethrin inhibition of [^{35}S]TBPS binding we studied the influence of deltamethrin on the kinetics of [^{35}S]TBPS binding in trout brain membranes. Deltamethrin modulation of [^{35}S]TBPS association and dissociation was examined in the presence and absence of 5 μM GABA. Brain membranes were subjected to three freeze-thaw cycles to remove endogenous GABA as previously described (Eshleman and Murray, Neuropharmacol., in press). The binding of [^{35}S]TBPS was performed at an incubation temperature of 12°C. In the absence of GABA, [^{35}S]TBPS association and dissociation are both adequately described by a single component model. In contrast, in the presence of GABA both association and dissociation reactions of [^{35}S]TBPS binding were better characterized by a two component model. In the presence of GABA the [^{35}S]TBPS association rate increases as a function of GABA concentration. Deltamethrin was found to affect [^{35}S]TBPS association in a GABA dependent manner. Deltamethrin effected a reduction in the rate constants for both the fast and slow components of association. In contrast, deltamethrin had no effect on the [^{35}S]TBPS dissociation rates. These results suggest that the mechanism of the apparent inhibition of equilibrium binding of [^{35}S]TBPS by deltamethrin is a reduction in the association rate of this reaction. (Supported by HHS Grant ES04891)

456.7

NORMALIZATION OF PCB - DEPRESSED CHAT ACTIVITY IN HIPPOCAMPUS AND BASAL FOREBRAIN OF YOUNG RATS BY THYROXINE INJECTION. L.A. Meserve, L.M. Juárez de Ku, T.A. Single*, D.M. Colon*, and M. Sharma*. Dept. Biol. Sci., BGSU. Bowling Green, OH 43403-0212.

Direct administration of polychlorinated biphenyl (PCB) to adult animals, and indirect provision to rat pups via the maternal diet, results in depression of thyroid status. Concomitant with hypothyroidism, young of rats fed PCB display subnormal activity of choline acetyltransferase (ChAT) in hippocampus and basal forebrain at 15 days of age. The present study was done to determine whether injection of thyroid hormone (thyroxine, T_4; or triiodothyronine, T_3) would normalize ChAT activity depressed by PCB. PCB (Arochlor 1254, 250 ppm) was incorporated into the maternal diet from day 1 of pregnancy until pups were 15 days old. Littermates were injected daily ip with either saline or replacement doses of T_4 (50 ng/g bw) or T_3 (10 ng/g bw). At 15 days of age, pups were decapitated, sera collected for T_4 and T_3 determination, and basal forebrains and hippocampi removed to measure ChAT activity. The previously demonstrated depression of ChAT activity by PCB to 33% of normal in hippocampus and 39% of normal in basal forebrain was confirmed. Injection of T_4 increased enzyme activity to 82% of normal in hippocampus and 90% of normal in basal forebrain. Injection of T_3 did not improve ChAT activity. The latter puzzling result may stem from the dosage of T_3 used or from alteration of accessibility of T_3 to the brain after T_3 exposure.

456.8

A WINDOW OF VULNERABILITY FOR DEVELOPING DOPAMINE RECEPTORS? M.H. Schmidt* and T. Lee. Psychopharmacology Unit, Clarke Inst. of Psychiatry, Toronto, Canada, M5T 1R8, and Dept. of Pharmacology, Univ. of Toronto, Toronto, Canada, M5S 1A8.

Prolonged prenatal blockade may impair ontogenic dopamine receptor acquisition in rat striatum (Rosengarten and Friedhoff, Science, 203: 1133, 1979; Scalzo et al., Pharmacol. Biochem. Behav., 34: 721, 1989). A window of vulnerability from embryonic day 15-18 has been described (Rosengarten et al., Birth Defects, 19: 511, 1983), but has not received recent attention.

To test the hypothesis that blockade during this brief period is sufficient to impair dopamine D_2 receptor acquisition, pregnant Wistar rats were given haloperidol, thiothixene or trifluoperazine at doses of 2.5, 5.0 or 7.5 mg/kg/day s.c. from gestational day 15-18. Pups were sacrificed 14 days after birth. Density and affinity of D_2 receptors in pooled striatal membranes of each litter were determined by Scatchard analysis, using [^{3}H]-spiperone ± sulpiride. In control tissues, density was found to be 116 ± 7 fmol/mg, while affinity was 0.18 ± 0.03 nM ($\bar{x}$ ± s.e.m.). The three drugs did not produce consistent, dose-dependent changes in D_2 receptor density or affinity.

The results of this study suggest that the brief period of exposure is insufficient to impair D_2 receptor ontogeny and call into question the concept of a window of vulnerability. [Supported by the Clarke Institute Research Fund and Ontario Graduate Scholarship Program (M.H.S.).]

456.9

IN VIVO ELECTROCHEMICAL STUDIES OF DOPAMINE DIFFUSION AND CLEARANCE IN THE STRIATUM OF NEONATAL 6-OHDA-LESIONED RATS. J. Luthman, M.N. Friedemann, B.J. Hoffer and G.A. Gerhardt, Depts. of Pharmacology and Psychiatry, University of Colorado Health Sciences Center, Denver, CO 80262.

Neonatal destruction of dopamine (DA)-containing neurons with 6-hydroxydopamine (6-OHDA) has been shown to produce rats that exhibit hyperactivity. However, few studies to date have explored the diffusional and cellular clearance of DA following these neonatal lesions which produce permanent damage to DA-containing neuronal systems. In the present study, high-speed (5 Hz) in vivo chronoamperometry was coupled with pressure ejection of DA from micropipettes to study the clearance and diffusion properties of DA. These measures were performed in the striatum of urethane anesthetized rats in both control rats and rats that had received neonatal (day 1) injections of 6-OHDA (75 micrograms intracisternal) following pretreatment with desipramine. In the 6-OHDA-treated animals, larger volumes of DA were needed to produce equivalent changes in the detected extracellular levels of DA. Temporal properties of DA signals were also seen to be greater in these rats. In addition, ejection parameters which were seen to produce detectable responses in the 6-OHDA-treated animals often produced no detectable signals in the striatum of control rats. These data suggest that neonatal 6-OHDA treatments change the clearance properties of DA by cellular elements. (Supported by USPHS grants AG06434, AG00441 and NS09199).

456.10

FETAL BRAIN CATECHOLAMINES AND INDOLEAMINES FOLLOWING REPEATED NITROUS OXIDE EXPOSURE. S.A. RICE, A. CARUGHI*, and M.T. SERRA*. Departments of Anesthesia, Stanford University and VA Medical Center, Palo Alto, CA 94304

Our previous work showed that repeated prenatal exposure of rats to nitrous oxide (N_2O) results in significant hyperreactivity of the acoustic startle reflex at 30 and 60 days of age (Teratology, Abs., in press). The present study was designed to determine if prenatal N_2O-exposure alters fetal brain catecholamines and indoleamines.

SD rats were exposed to 35% N_2O or air for 4 hr on days 6-15 of pregnancy. 24 hr following the last exposure, rats were killed and fetuses were removed. Brains of 3 fetuses from each litter were pooled (N=6 litters/group); extracted into .1 M acetate buffer, pH 3.5, containing .3 mM EDTA; and analyzed by HPLC with E.C. detection.

Prenatal N_2O exposure significantly decreased brain norepinephrine, epinephrine and MHPG content compared to air-exposed fetuses (110 ± 29 vs. 180 ± 48 fmol/mg; 152 ± 47 vs. 228 ± 54 fmol/mg; and 24 ± 10 vs. 52 ± 10 fmol/mg, respectively). Brain 5-HIAA and HVA contents were significantly increased in N_2O-exposed compared to air-exposed fetuses (460 ± 104 vs. 289 ± 25 fmol/mg; and 1921 ± 750 vs. 719 ± 171 fmol/mg, respectively). Brain dopamine, DOPAC and serotonin contents were not different, nor were fetal weight, protein content or DNA content. [(Supported by VA Medical Center, Palo Alto, CA)]

456.11

CNS EFFECTS OF 5-HYDROXYTRYPTAMINE RELATED OXIDATION PRODUCTS, C. L. BLANK, R. N. GOYAL*, M. WRONA*, G. DRYHURST* AND D.J. TURK, Dept of Chemistry and Biochemistry, U. of Oklahoma, Norman, OK 73019.

A variety of unique compounds has been obtained as the result of the investigation of the oxidative behavior of 5-hydroxytryptamine (5-HT) and related indoleamine species. Some of these compounds have exhibited substantial toxic effects when administered intracerebrally in mice. Some have led to interesting behavioral reactions following such administration. In order to establish an appropriate dose at which to examine the effects of these species on endogenous neurochemical levels, preliminary investigations examined the LD_{50} values for each using the Dixon Up-Down method (W.J. Dixon, *J. Am. Stat. Assoc.*, **60**, 967, 1965). The results obtained were:

Compound	LD_{50}, µg
5-hydroxytryptamine-4,7-dione	30 ± 1
7-S-(glutathionyl)-tryptamine-4,5-dione	21 ± 1
6,6'-bi-(5-hydroxytryptamine-4,7-dione)	25 ± 2
5,7-dihydroxytryptamine	52 ± 1
2,7'-bi-(5,6-dihydroxytryptamine)	> 125
7,7'-bi-(5-hydroxytryptamine-4-one)	> 125

Endogenous levels of catecholamines, indoleamines, and acetylcholine, as well as related metabolites, were determined using a NEUBA® Neurobiological Analyzer. This system provides valuable qualitative information involved in the identification of the pertinent species through the use of multiple amperometric electrochemical detectors. It provides rapid throughput by using a multicolumn liquid chromatographic setup. Behavioral effects observed for the compounds listed above included hyperexcitability, sedation, short-term (72 hr) partial motor impairment, and rapid "rolling over."

456.12

NEUROTOXIC EFFECTS OF COCAINE AND METHAMPHETAMINE ON CULTURED DOPAMINE NEURONS. BA Bennett, JE Clodfelter*, M Morris, DK Sundberg* and CR Miller*. Dept. of Physiology and Pharmacology, Bowman Gray Sch. Med. of Wake Forest Univ., Winston-Salem, NC 27103

Stimulant drugs of abuse produce alterations in movement and behavior associated with histological and biochemical effects on mesencephalic (MES) dopamine (DA) neurons while the effects on the tuberoinfundibular DA system of the medial basal hypothalamus (MBH) are not known. These dopaminergic neurons differ from those in the MES by lacking a high affinity uptake system and D2 autoreceptors. This study examined the effects of stimulant agents in cultures of MES and MBH using immunocytochemical methods.

Brains from neonate rats (1-2 days old) were removed and the MES and MBH obtained and dispersed into single cells. Stimulant agents, either cocaine (COC) or methamphetamine (METH; 10^{-3}-10^{-9}M), were added to the cultures on day 2. Cultures were fixed on day 7 with 5% acrolein. The antibodies used were tyrosine hydroxylase (TH) and neuron specific enolase (NSE). Tissues were processed according to the Vectastain protocol. Computerized morphometrics were used for analysis.

There was a generalized neurotoxicity observed with COC and METH (10^{-3}M) as demonstrated by a dramatic decrease (>90%) in both NSE and TH positive cells. With concentrations of 10^{-5} and 10^{-7}M, there was a specific decrement in TH positive neurons with both drugs, but there was a greater decrement in the MES cultures. The effects of COC and METH were very similar. In the presence of 10^{-5} and 10^{-7}M drugs, MBH TH positive neurons were reduced 35% and 15%, respectively. Likewise, MES TH positive cells decreased 50% and 30%, respectively, in the presence of 10^{-5} and 10^{-7}M drugs. Our findings are consistent with previous studies examining the effects of METH on MES cultures (Kontur et al, Dev Br Res 31:7,1987). There was no significant change observed with 10^{-9}M. These results indicate that COC and METH have specific neurotoxic effects on both MES and MBH dopamine neurons. Supported by grant DA05073 (BAB).

456.13

MATERNAL TOXICITY AND OFFSPRING DEVELOPMENT FOLLOWING GESTATIONAL EXPOSURE TO COCAINE IN THE RAT. L. M. Donohue*, L. A. Freed, H. E. Hughes and D. L. Dow-Edwards, Laboratory of Cerebral Metabolism, Department of Neurosurgery, SUNY-Health Science Center, Brooklyn, N.Y. 11203.

Clinical and animal studies indicate that prenatal cocaine exposure results in maternal toxicity and neurobehavioral abnormalities in the offspring. The current study investigated the effects of prenatal cocaine exposure on several maternal and fetal indices of toxicity in the rat. Pregnant Sprague-Dawley rats were gastrically intubated with 30 or 60 mg/kg/day cocaine-HCl or vehicle during gestational days (GD) 8-22. Vehicle treated rats were pair-fed/watered to rats receiving the higher dose of cocaine. A non-treated control group was also maintained. Daily maternal body weights and food/water consumptions were recorded during GD 8-22. At parturition, dams were sacrificed and the number of implantation sites recorded. The occurrence of abruptio placentae was also noted. Pups were weighed, sexed, culled to 10 and surrogate fostered. Onset of the following developmental milestones was assessed daily in the offspring: pinna eruption; eye opening; crawling; walking; bar grasp; righting and negative geotaxis. Additional pups were sacrificed at birth to determine the weights of brain, thymus, adrenal gland and kidney. Preliminary data indicate a delay in crawling onset for males and a reduction of adrenal weight in females in the 60mg/kg cocaine group compared to the non-treated group. Maternal toxicity data are forthcoming.

Supported by ADAMHA Grant #DA04118.

456.14

EXPOSURE TO TRIMETHYLTIN INCREASES CHOLINE ACETYLTRANSFERASE ACTIVITY IN THE RAT HIPPOCAMPUS Cannon R.L.+, Hoover, D.B.++, and Woodruff, M.L.+ Dept. of Anatomy+ and Pharmacology++, J. H. Quillen Col. of Med., East Tenn. St. U., Johnson City, TN. 37614

Trimethyltin (TMT) damages all parts of the hippocampus, but also enhances acetylcholinesterase (AChE) staining in the dentate gyrus of rats. The purpose of this experiment was to verify TMT-induced enhancement of the cholinergic projection to dentate using a biochemical assay quantifying choline acetyltransferase (ChAT) activity in the dentate. Five male hooded rats were gavaged with 7mg/kg TMT chloride while 5 served as controls. One hundred days later the rats were killed and serial frozen sections were taken of the brain. Micropunch samples were taken from 300 µm sections at three rostral-caudal levels of the dentate gyrus and CA1 of the hippocampus. Micropunches of the caudate-putamen (c-p) were also taken. ChAT activity was measured on all samples. Alternate sections were stained for AChE or with thionin. Inspection of these sections revealed histological changes consistent with previous reports. ChAT activity did not differ between controls and TMT-treated rats for c-p samples, but a significant increase in ChAT was found in TMT-treated rats for all levels of the dentate gyrus and CA1. These results indicate that exposure to TMT increases cholinergic innervation of both the dentate gyrus and the CA1 portion of the hippocampus. (Supported by a grant from the NIH (ES04070-04) to MLW.)

456.15

MOLECULAR CLONING AND ANALYSIS OF mRNA EXPRESSED IN TRIMETHYLTIN-SENSITIVE NEURONS. S. M. Toggas*, J. K. Krady*, J. W. Polli* and M.L. Billingsley. Department of Pharmacology and Center for Cell and Molecular Biology, Penn State University College of Medicine, Hershey, PA 17033.

Trimethyltin (TMT) is a selective neurotoxicant that destroys neuronal subpopulations which have no apparent neuro-chemical or anatomic relationships. To isolate gene products common to sensitive neurons, a cDNA subtraction library was created using avidin/biotin-based methods. One clone, pr9T19, gave patterns of hybridization in Northern blot and *in situ* hybridization experiments suggesting that it was expressed in TMT-sensitive neurons. Strong hybridization was seen in cingulate and piriform cortex and hippocampus. pr9T19 was expressed in rat telencephalon on embryonic day 15; the pattern of expression became restricted to the adult pattern by postnatal day 20. *In situ* hybridization in human hippocampus suggested that an mRNA related to pr9T19 was expressed in subpopulations of hippocampal pyramidal cells. High stringency Southern blot analysis indicated that a related gene was present in drosophila, rabbit, rat, and human genomic DNA. Initial sequence analysis suggests that the 3.0 kb mRNA encodes a novel protein of 123 amino acids. Experiments are underway to characterize this protein and to determine its possible role in the neurotoxicology of TMT.

DEGENERATIVE DISEASE—OTHER: BASAL GANGLIA

457.1

PROGRESSION OF BASAL GANGLIA ATROPHY AND FUNCTIONAL DECLINE IN HUNTINGTON'S DISEASE PATIENTS. J.K.A. Roberts and E.A. Loh. Dept. of Neuropsychiatry, Royal Ottawa Hospital, Ottawa, Canada.

Neuroanatomical and functional changes associated with the progression of Huntington's disease (HD) were investigated. CT scans were obtained from 14 individuals at various stages of HD. Scans were digitized, measures of 1) distance between frontal horns of lateral ventricles (FH), 2) distance between caudate nuclei (BICAUD), and 3) width of globus pallidus and putamen regions (GP) were calculated. These measures were compared with patient's scores on the Quantitative Neurological Examination (QNE), Shoulson and Fahn's functional assessment (S&F), and Mini-Mental State (MMS) scales. Measures of the duration of HD and the GP and BICAUD regions were significantly correlated (r's > .70). Further, the GP measure was sig. correlated with MMS and (r =.90) and S&F (r= -.81) scores. Measures more specific to the caudate regions: FH and BICAUD were more strongly related to the motor function score QNE (r > .85). This indicates that atrophy of the GP region may be a better index of functional deterioration than the more commonly used measures of caudate atrophy which were more closely related to motoric decline.

457.2

CLINICAL CORRELATES OF THE VONSATTEL SCORE IN HUNTINGTON'S DISEASE. CE Peyser, SE Folstein, RM Zweig and JC Hedreen. Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205.

Prominent features of Huntington's disease (HD) include a movement disorder and progressive dementia. The most consistent neuropathological changes are neostriatal atrophy and neuronal loss; Vonsattel et al. (1985) developed a widely used grading system based on these changes. The purpose of this study was to examine clinical correlates of the Vonsattel score in well characterized HD cases. For 36 HD patients examined $\leq$ 18 months before death, mean scores on clinical variables for patients with Vonsattel gradings (0+1,2,3,4) were obtained; Spearman rank-order correlations were determined for relationship between Vonsattel severity and the following clinical variables: Mini Mental State Exam (dementia measure; r_S=-.52, p=.001); Quantitative Neurological Exam Score (r_S=.67, p .001); Motor Impairment Score (voluntary motor abnormality score; r_S=.71, p .001); and Activity of Daily Living Scale (functional impairment measure; r_S=.49, p=.002). Similar results were obtained for 42 HD cases with longer intervals between clinical measures and death. We conclude that the Vonsattel score in HD is a powerful measure of disease severity as indicated by its high correlation with extent of neurological, cognitive and functional impairment.

457.3

PATHOLOGICAL CHANGES IN EARLY HUNTINGTON'S DISEASE. J.C. Hedreen. Neuropathology Laboratory, The Johns Hopkins University School of Medicine, Baltimore, MD 21205.

The distribution of histopathological changes in autopsied cases of Huntington's disease (HD) with different pathological grades of severity was examined by means of glial fibrillary acidic protein immunocytochemistry and counts of neurons and astrocytes. Two principal findings were made. First, in three presumed grade-0 HD cases, it was determined that neuronal counts in the dorsal putamen were less than counts in controls; this finding provides a significant aid to diagnosis in very mildly affected cases. Second, in many grade-2 and grade-3 cases, a nonuniform pattern of pathology was seen at the ventrally advancing margin of pathological change. In this region, areas with neuronal cell loss and gliosis were interspersed with relatively normal areas, forming patterns reminiscent of those created by the intrinsic chemoarchitecture of the neostriatum and by striatal afferents. This finding suggests that neuronal injury in HD may initially involve neurochemically specific cell groups in the striatum or striatal zones innervated by specific afferent systems. Identification of these specific cell groups or afferents will have important implications for understanding the mechanism of neuronal injury in HD.

457.4

LOCUS COERULEUS PATHOLOGY: A FEATURE OF ADVANCED HUNTINGTON'S DISEASE. R.M. Zweig, C.A. Ross, J.C. Hedreen, C.E. Peyser*, J.E. Cardillo*, S.E. Folstein* and D.L. Price. Univ. of Nevada Sch. of Med., Reno and Johns Hopkins Hosp., Baltimore.

Numbers of neurons at up to three strictly defined anatomical levels of the locus coeruleus (LC) were determined in 33 patients with Huntington's disease (HD) and in 22 age-matched controls. Distances between rostral and caudal levels (LC length) could be determined in 23 of the HD and in 7 of the control cases. Rostral level cell counts were significantly less in HD patients who had severe dementia than in controls and, at rostral and middle levels, in HD patients with severe dementia compared to those with mild dementia. Fewer rostral and middle level LC neurons correlated with neostriatal atrophy and last recorded motor impairment (MIS) and activities of daily living (ADL) scores. LC length was reduced by >20% in patients with severe dementia compared to those with mild dementia (p=.002) or controls. Reduced LC length correlated with early age of onset, long duration, MIS, and most highly, ADL score (Pearson r=0.72; p<.001). In conclusion, LC pathology, especially as reflected in LC length, correlates highly with clinical features of advanced disease.

457.5

EXCITATORY AMINO ACID RECEPTOR POPULATIONS IN HUNTINGTON'S DISEASE L.S. Dure IV, J.B. Penney, A.B. Young. Department of Neurology, University of Michigan, Ann Arbor, MI 48109 U.S.A.

Huntington's Disease (HD) is an autosomal dominant neurodegenerative disease with onset of dementia and involuntary movements in mid-life. Although the pathogenesis of HD is unknown, the inherent neurotoxicity of excitatory amino acids (EAA) has been implicated. One subtype of EAA receptor, the N-methyl-D-aspartate (NMDA) receptor, has been shown to be decreased in the basal ganglia of HD brains. Using *in vitro* quantitative receptor autoradiography to determine EAA receptor population densities, we studied the binding characteristics of EAA and associated receptors in 15 brains of patients with HD, and 16 control brains. Receptor assays for NMDA-sensitive [^{3}H]glutamate, [^{3}H]glycine (which binds to a putative modulatory site on the NMDA receptor), [^{3}H]MK-801 (which binds to the phencyclidine (PCP) site of the NMDA receptor), [^{3}H]AMPA(α-amino-hydroxy-5-methyl-isoxazole propionic acid), quisqualate-sensitive (metabotropic) [^{3}H]glutamate, and [^{3}H]kainate binding all showed marked decreases when compared to controls. There was a greater loss of NMDA-sensitive [^{3}H]glutamate and [^{3}H]kainate binding in HD compared to the losses of other receptor populations. These data supports the hypothesis that an excitotoxic mechanism mediated by subpopulations of glutamate receptors contributes to the pathology of HD.

Supported by the Huntington's Disease Society of America (LSD) and USPHS grant NS 15655 (JBP and ABY).

457.6

THE KYNURENINES: NEUROTOXINS WITH A POTENTIAL ROLE IN HUNTINGTON'S DISEASE (HD). G.K. Rieke and B. Kurunwune*. Dept. Anat. & Cell Biol., Meharry Med. College, Nashville, TN 37208.

3-Hydroxykynurenine (3HKYN) and L-Kynurenic acid (L-KYN) brain levels are increased 2-3 fold in HD. To satisfy the neurotoxic hypothesis for HD these endogenous compounds must be neurotoxic. We have tested the neurotoxicity of L-KYN, 3HKYN and related metabolites. A cannula coupled to an Alzet osmotic pump was implanted into the striatum. Each pump was filled with buffered L-KYN or quinaldic acid (0.31 ug/uL), or 3HKYN (3.36 ug/uL). L-KYN and 3HKYN produced selective neuron sparing lesions, similar to pathology seen in HD. NADPH diaphorase positive neurons were present in the spongiose zone of the L-KYN and 3HKYN induced lesion. Quinaldic acid, the terminal metabolite of L-KYN, was not neurotoxic. The neurotoxic potential of kynurenine is unknown, while the role of the neurotoxin quinolinate in HD is doubtful. L-KYN and 3HKYN are endogenous neurotoxins. Their role in HD, along with other neurotoxins like pyroglutamate, must be carefully examined. The presence of a specific neurotoxin in specific HD families may reflect subtle errors within the HD gene locus. Supported by NIH S06RR08037.

457.7

MET-ENKEPHALIN, SUBSTANCE P, AND GABA LEVELS IN RIGID AND CHOREIC HUNTINGTON'S DISEASE PATIENTS. E. Storey and M.F.Beal. Neurochemistry Laboratory, Mass. Gen. Hosp., Boston, MA 02114.

It has recently been suggested on the basis of immunohistochemical studies that chorea in Huntington's disease (HD) results from early depletion of met-enkephalin (ME)-containing spiny GABAergic neurons projecting to the external segment of the pallidum (GPE), with initial relative sparing of substance-P (SP)-containing GABAergic neurones projecting to the internal segment of the pallidum (GPI). The imbalance between these opposing pathways is thought to result in disinhibition of the ventrolateral thalamus, and increased excitatory input to the cortex, resulting in chorea. In an attempt to confirm this hypothesis, brains from 9 adult choreic HD patients were compared with 9 adult and 6 juvenile-onset rigid cases,and with 12 control brains. All patients were examined clinically within 6 months of death. All but one of the affected brains were grade 3 or 4. There were significant differences in SP, ME, and GABA levels in grade 3 versus grade 4 cases. However, measurements of SP and ME levels by RIA failed to show any preferential preservation of SP versus ME in choreic cases, and indeed ME was better preserved than SP in the striatum. Measurement of GABA levels by HPLC also failed to show preferential preservation in the GPI in cases with chorea versus those with rigidity. The findings were unchanged when only choreic (6) and rigid (5) grade 3 cases were compared. Depletions of SP and GABA in the GPI were not significantly different from depletions of ME and GABA in the GPE when all cases were combined. There was, however, a trend towards relative preservation of GPI SP and GABA in choreic patients. Although a preferential loss of ME-containing GABAergic projection neurones to the GPE may play a role in the genesis of chorea, these results did not demonstrate this in advanced cases.

457.8

TERMINAL STRIATAL SUBSTANCE P- AND MET-ENKEPHALIN-PROJECTIONS IN THE GLOBUS PALLIDUS ARE EQUALLY AFFECTED IN HUNTINGTON'S DISEASE. **R.J. Ferrante, N.W. Kowall, K. Harrington, E.P. Richardson, Jr., Mass. Gen. Hosp. Boston, MA 02114**

Spiny striatal projection neurons are most severely involved in Huntington's disease ((HD). A major subcortical target of these neurons is the globus pallidus (GP). Striatal terminal projections to GP have been identified with a variety of neurochemical markers. We stained contiguous, 50 μm-thick sections of the GP in 17 neurologically normal control and 16 HD brains for substance P (SP), methionine enkephalin (ME), and transforming growth factor alpha (TGF), an amino acid peptide which extensively colocalizes with ME. The HD brains were from cases of low to very severe grades (G0-G4), all adult onset. In both controls and HD, the external pallidal segment (GPe) reacted primarily for ME and TGF with little terminal staining occurring in the internal segment (GPi). SP was primarily located within the GPi. Intense, terminal SP-activity rimmed the GPe with scattered fiber staining within. In HD there was a gradient of SP-, ME-, and TGF-loss in the GP corresponding to the severity of neuropathologic change, with the most marked reductions occurring in the very severe grades. No significant differences in the pattern and relative intensity of SP-, ME-, and TGF-terminal activity was observed in any HD case. Photodensitometric image analysis supported these findings. The density of staining in HD cases, measured as a percent of the normals, was nearly equal for SP, ME, and TGF in each grade (G0=96.2%, G1=85.0%, G2=73.7%, G3=40.2%, G4=9.0%). Our findings suggest that spiny striatal projection neurons are equally involved in HD.

457.9

MUSCARINIC RECEPTORS IN BASAL GANGLIA OF PROGRESSIVE SUPRANUCLEAR PALSY (PSP). N. S. Hermanowicz, J. B. Penney, N. L. Foster, I. Shoulson. Depts. of Neurology, University of Michigan, Ann Arbor, MI 48104-1687 and University of Rochester, Rochester, NY 14642

PSP is characterized by severe nigral, subthalamic, superior collicular and pallidal atrophy with lesser striatal pathology. Choline acetylase and dopamine D2 receptors are reported to be decreased in striatum (Ruberg et al. *Ann. Neurol.* 18:523,1985). We have measured muscarinic cholinergic receptors using quantitative autoradiography in basal ganglia of 8 PSP brains and compared them to 11 control, 9 Huntington's (HD) and 7 Parkinson's (PD) disease brains.

Whole coronal, cryostat sections through medial globus pallidus were incubated at 20°C for 3 hrs. in 1 nM [^{3}H]QNB (Amersham) in 50 mM Tris-HCl. Sections were exposed to Ultrofilm-3H (LKB) for 2 weeks and analyzed with an MCID (Imaging Research). Binding in pm/mg protein (mean±sem) was:

Disease	Caudate	Putamen	Lateral Globus Pallidus	Medial Globus Pallidus
Control	2.30±.30	2.17±.25	.207±.016	.236±.021
PSP	2.06±.14	1.45±.11*	.256±.036	.538±.172*
HD	.822±.092*	.875±.079*	.096±.011*	.079±.009*
PD	2.27±.14	2.16±.17	.219±.022	.296±.061

(*, p<.05 by ANOVA with post hoc pairwise comparisons to control)

In HD striatal cell loss is much more severe than pallidal. Thus, the decreased pallidal QNB binding in HD suggests that these binding sites are on striatal efferent terminals. Decreased putamenal QNB binding in PSP indicates a loss of cells bearing muscarinic receptors in putamen. The increased medial pallidal binding suggests a concentration phenomenon due to the known pallidal cell loss in PSP. These pallidal QNB binding sites in PSP may be on a spared subset of striatal efferent terminals. Supported by USPHS grant AG08671.

457.10

ABNORMALITIES OF DOPAMINERGIC MARKERS IN POSTMORTEM STRIATAL SPECIMENS FROM PATIENTS WITH TOURETTE SYNDROME Harvey S. Singer, In-Hei Hahn, Timothy H. Moran. The Johns Hopkins University School of Medicine. Baltimore, Maryland 21205.

The dopamine hypothesis for Tourette Syndrome (TS) was evaluated in frozen postmortem striatal tissue from three adults (2 males, 1 female) with the diagnosis of TS and up to 13 controls. [^{3}H]Mazindol binding (fmol/mg protein), used to label the DA uptake carrier site, was significantly increased in the striatal region of patients with TS (caudate: TS = 467 ± 22, control = 342 ± 28; putamen: TS = 604 ± 37, control = 404 ± 43). HPLC measurements of dopamine and its metabolite DOPAC were normal to slightly reduced. [^{3}H]SCH 23390 binding, labels the D1 receptor, was not significantly different from controls in either caudate (TS = 39.5 ± 9.4, control = 50.1 ± 7.4) or putamen (TS = 52.8 ± 4.8, control = 43.2 ± 5.5). D2 receptor binding, measured by [^{3}H]spiperone, was slightly greater in caudate (TS = 69.1 ± 12.5, control = 58.9 ± 8.4) and putamen (TS = 39.3 ± 5.3, control = 32.3 ± 2.2).

Our data supports earlier proposals of a dopaminergic abnormality within the basal ganglia. However, rather than a specific dysfunction of postsynaptic D1 or D2 receptors, results from postmortem analyses suggest a significant alteration of DA uptake mechanisms.

457.11

INTRAREGIONAL PATTERN OF STRIATAL CHOLINERGIC ENZYME REDUCTION IN DOMINANTLY-INHERITED OLIVOPONTOCEREBELLAR ATROPHY: POSSIBLE RELATIONSHIP TO FRONTAL LOBE SYSTEM IMPAIRMENT. S.J. Kish, M. El-Awar*, L. Schut*, M. Oscar-Berman*, Y. Robitaille*, J. Deck*, L. DiStefano*, L.J. Chang*, and M. Freedman*. Clarke Inst. Psychiat., Rotman Research Institute of Baycrest Centre, Toronto, Canada; U. Pittsburgh; VA Hosp., Boston/Minneapolis.

We measured the intraregional pattern of the activity of the cholinergic enzyme cholineacetyltransferase (ChAT), the specific cholinergic marker, in striatum of six patients from one family with dominantly-inherited olivopontocerebellar atrophy (OPCA). Previous neuropsychological testing of affected members of this family, including the three patients tested in this study, revealed signs of frontal lobe impairment. As compared with the controls (n=11), mean activity of ChAT was severely reduced in OPCA throughout the subdivisions of the caudate head nucleus with the dorsal portion (-81 to -85%) being the most affected. In contrast, ChAT levels in the putamen were much less markedly reduced (-26 to -61%). In view of the experimental and clinical evidence indicating that the caudate nucleus may be a critical component of pre-frontal cortical-governed behaviour, we suggest that the severe cholinergic reduction in caudate nucleus of our OPCA patients may contribute to the frontal system impairment observed in this disorder. (Supported by U.S.NIH #NS26034.)

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SYMPOSIUM: REGULATION OF NICOTINIC ACETYLCHOLINE RECEPTOR EXPRESSION AND FUNCTION. R.J. Lukas, Barrow Neurological Institute (Chairperson); R.L. Huganir, Johns Hopkins Univ. Sch. of Med.; J.P. Merlie, Washington Univ.; D.K. Berg, Univ. of Calif. San Diego; T. Claudio, Yale Univ.

Nicotinic acetylcholine receptors (nAChR) are structurally and functionally diverse members of the ligand-gated ion channel superfamily of multi-subunit transmembrane proteins. nAChR diversity is partly based on heterogeneity of nAChR subunit-encoding genes and may provide teleologically beneficial functional and regulatory flexibility to a plastic and dynamic nervous system. The participants will address this thesis and will show how contemporary multidisciplinary approaches are yielding insight into cellular and molecular mechanisms that regulate nAChR expression and function. Lukas will provide an overview of nAChR biology and a synposis of studies on muscle and neuronal nAChR regulation in model clonal cell lines. Huganir will describe studies on regulation of muscle-like nAChR processing and functional characteristics by serine and tyrosine protein kinases. Merlie will address transcriptional regulation of receptor expression in muscle with specific reference to promoter analysis in transgenic mice. Berg will review work on regulation of neuronal nAChR function and number in autonomic ganglia. Claudio will discuss studies on synaptogenesis and biogenesis of muscle-like nAChR in transfected cells.

460

SYMPOSIUM: Neuronal regulation of renal function: A model system for nervous system interactions. J.M. Wyss, Univ. of Alabama at Birmingham (Chairman); L. Barajas*, UCLA; U.C. Kopp, Univ. of Iowa; L.P. Schramm Johns Hopkins Univ.; J. Ciriello, Univ. Western Ontario.

The kidney is the most highly innervated peripheral organ, and the relative simplicity of the neural-renal interaction makes this system an important model for future research into all neural-peripheral and neural-neural interactions. *L. Barajas* will examine the intrarenal distribution of the efferent renal nerves along the nephron and vasculature, and the localization of afferent nerves within the kidney. *U.C. Kopp* will discuss the role of the renorenal reflex, which alters renal responses following stimulation of the contralateral kidney. She will also consider the finding that efferent renal nerve activity directly modifies sensory feedback to the spinal cord from the kidney. *L.P. Schramm* will discuss his studies into spinal and supraspinal control of renal function, which have led to an elucidation of inhibitory networks within the spinal cord that regulate the function of peripheral organs. He also will discuss his recent use of pseudorabies virus to localize the preganglionic nerves that project to the kidney. *J. Ciriello* will elucidate the brain mechanisms that contribute to the regulation of renal function and will examine the ability of the kidney (via renal nerves) to modify the activity of the neurons in the brain. *J.M. Wyss* will examine the functional consequence of neural control of the kidney in health and disease. Although the nervous system often has been considered as only an acute regulator of visceral function, current studies into hypertension and renal disease suggest that neural-renal dysfunction may be an important contributor to chronic diseases.

EXCITOTOXICITY V

461.1

FOCAL INJECTION OF AMINOOXYACETIC ACID PRODUCES SEIZURES AND LESIONS IN RAT HIPPOCAMPUS: EVIDENCE FOR MEDIATION BY NMDA RECEPTORS. O.G. McMaster, F. Du, E.D. French[1] and R. Schwarcz. Md. Psych. Res. Ctr., Baltimore, MD 21228, and [1]Dept. Pharmacol., Univ. Ariz. Coll. Med., Tucson, AZ 85724.

Aminooxyacetic acid (AOAA), known to produce both convulsant and anti-convulsant effects in rodents upon systemic administration, was recently found to powerfully inhibit kynurenine aminotransferase, the enzyme responsible for the production of the excitatory amino acid receptor antagonist kynurenic acid (KYNA). We have now used AOAA as a tool to examine the effects of a reduction of KYNA levels in vivo. Intrahippocampal application of AOAA to unanesthetized rats caused dose-dependent seizure activity which was monitored and quantified by EEG. Co-administration of the selective NMDA receptor antagonist D-APH (45 or 225 nmoles) with 225 nmoles AOAA resulted in almost complete abolition of seizure activity. Rats receiving 45 or 225 nmoles AOAA also suffered selective loss of CA1 pyramidal neurons. AOAA-induced neurotoxicity, too, was apparently mediated by NMDA receptors since it was attenuated by co-injection of 45 or 225 nmoles D-APH. Iontophoretic application to CA1 pyramidal cells (N=10) did not reveal any evidence for direct NMDA receptor activation by AOAA. AOAA may therefore precipitate excitotoxicity and seizures indirectly by removing tonic inhibition of KYNA at the NMDA receptor. (Supported by USPHS grant NS 16102).

461.2

EFFECT OF STRIATAL QUINOLINATE LESIONS ON KYNURENINE AMINOTRANSFERASE IN THE RAT BASAL GANGLIA. W. Schmidt, F. Du, E. Okuno and R. Schwarcz. Maryland Psychiatric Research Center, Baltimore, MD 21228.

Kynurenine aminotransferase (KAT), the enzyme responsible for the production of kynurenic acid (KYNA), was studied in the striatum and substantia nigra in normal rats, and in animals 2 and 7 days following a unilateral intrastriatal injection of quinolinic acid (QUIN; 50 μg/1 μl). In normal striata, immunocytochemical studies with anti-rat KAT antibodies revealed a preferential astrocytic localization of the enzyme and a small population of KAT-positive neurons, which was dispersed throughout the striatum. 7 days after QUIN injection, the number of KAT immunoreactive astroglial cells in the striatum was markedly increased as compared to the contralateral side.

QUIN-lesioned striata (glutamate decarboxylase activity: -71.6±4.7% and -87.2±2.0% after 2 and 7 days, respectively) showed a 19.9±2.1% reduction in KAT activity after 2 days and a 186.5±9.8% increase in enzyme activity after 7 days (N=8 each). The early decrease is probably due to the loss of KAT-containing neurons while the later increase may be the result of astrocytic proliferation. KAT activity in the substantia nigra ipsilateral to the lesion was unchanged after 2 days but increased to 141.3±19.1% of controls after 7 days (N=6 each).

Supported by USPHS grant NS 28236.

461.3

EXCITOTOXIC AND NON-EXCITOTOXIC DAMAGE PRODUCED BY 3-HYDROXYANTHRANILIC ACID IN RAT CORTICOSTRIATAL CULTURES. W.O. Whetsell, Jr., B. Christie-Pope, and R. Schwarcz[1]. Vanderbilt Univ., Nashville, TN 37232-2561 and [1]Md. Psych. Res. Ctr., Baltimore, MD 21228.

3-hydroxyanthranilic acid (3HANA) is a brain metabolite and bioprecursor for the excitotoxic agent, quinolinic acid (QUIN). Mature (21 days *in vitro*) organotypic cultures of rat corticostriatal system were incubated in 3HANA (5mM) alone or concomitantly with the NMDA receptor antagonist, D-APH (1mM). Ultrastructural changes were assessed for up to 50 hours of incubation. At 10 hours, cultures exposed to 3HANA alone began to develop post-synaptic swelling and occasional dendritic swelling away from synaptic complexes. By 20 hours, generalized dendritic and axonal swelling was severe and even some neuronal swelling was evident; by 40 hours, cultures were destroyed by the treatment. In contrast, simultaneous incubation with 3HANA and D-APH induced no appreciable ultrastructural change in the cultures until 30 hours when there was scattered non-specific swelling of elements in the neuropil; no distinct post-synaptic swelling could be identified. By 50 hours, wide-spread swelling of neuropil and neurons was evident, but dendrites, axons and neurons were still recognizable. These studies suggest that 3HANA can cause toxic damage by two distinct mechanisms: one appears to be excitotoxic in character, possibly via *in vitro* production of QUIN, and antagonized by D-APH while the other may reflect oxidative cell destruction. (Supported by USPHS Grant NS-28236.)

461.4

SELECTIVE SIGMA LIGANDS PROTECT AGAINST DYNORPHIN A-INDUCED SPINAL CORD INJURY IN RATS. J.B. Long, R.E. Tidwell*, F.C. Tortella, K.C. Rice[1], and B.R. deCosta[1]*, Neuropharm. Br., Dept. of Med. Neurosci., Walter Reed Army Inst. of Res., Washington, D.C. 20307 and [1]Lab. of Med. Chem., NIDDK, Bethesda, MD 20892.

Lumbar spinal subarachnoid injection of dynorphin A (DYN) causes ischemia, neuronal degeneration and persistent hindlimb (HL) paralysis in rats. Excitatory amino acids have been implicated as mediators of DYN-induced spinal cord injury due to the protective effects of competitive and noncompetitive NMDA receptor antagonists, including MK-801, dextrorphan, and dextromethorphan (DM). However, recent evidence suggests that protective effects of DM in this model might involve binding sites in addition to those associated with the PCP/NMDA receptor complex. DM binds to high affinity sites which are quite similar to the sites identified by prototypic sigma (σ) ligands such as (+)-PPP. To address the involvement of DM/σ binding sites in neuroprotective mechanisms, we evaluated the effects of several highly σ selective substituted phenylethylamines (BD 737, BD 738, BD 1008, and BD 1063) on recovery from the persistent motor deficits caused by L4-L5 subarachnoid injections of 20 nmoles of DYN. Immediate preinjection of these compounds (50-200 nmoles, i.t.) failed to block the HL paralysis acutely induced by DYN; however all 4 σ compounds caused significant persistent improvements in HL neurological scores by 24 hr postinjection. These results indicate a potential usefulness of σ receptor ligands in the treatment of CNS injury.

461.5

MK801 INDUCES THE 70kD HEAT SHOCK PROTEIN AND FOS IN THE CINGULATE GYRUS. James W. Sharp, Stephen M. Sagar, and Frank R. Sharp, Depts. Neurology and Physiology, Univ. Calif. and VA Medical Center, San Francisco, CA. 94121

MK801, a non-competitive NMDA receptor blocker, has been shown to protect the nervous system from injury both *in vivo* and *in vitro*. However, recent studies suggest that this drug may selectively kill some neurons (Olney et al., Science *244*:1360; Allen et al., Science *247*: 221) in cingulate cortex.

Because we have proposed that heat shock gene expression (Gonzalez et al., Mol. Brain Res. *6*:93) may be a useful marker of stressed neurons, we have examined the effects of MK801 on HSP70 expression. 1mg/kg of MK801 given intraperitoneally induced HSP70 and Fos, detected immunocytochemically, in many neurons in cingulate and retrosplenial areas of cortex 18h after administration. This is a dose reported to produce reversible vacuolization of these neurons. The effects of 5mg/kg of MK801, a dose reported to produce irreversible neuronal injury in cingulate cortex, is currently being investigated.

The data support the usefulness of HSP70 in identifying cells stressed by a variety of insults. This and other data suggests that cells stressed by various insults that are destined to survive express both HSP70 mRNA and protein, whereas lethally injured cells may or may not express the protein.

461.6

ANTICHOLINERGICS PREVENT NEUROTOXIC SIDE EFFECTS OF NMDA ANTAGONISTS J.W. Olney, J. Labruyere*, G.J. Wang, M.T. Price. Washington University Medical School, St. Louis, MO 63110.

Although antagonists of the N-methyl-D-aspartate (NMDA) subtype of glutamate receptor are potentially useful for preventing neuronal degeneration in certain neurological disorders, treatment of adult rats with these compounds results in neurotoxic side effects consisting of pathomorphological changes in certain neurons of the cingulate and retrosplenial cerebral cortices (Olney et al., Science, 1989). Following low doses, these changes may be reversible, but higher doses can result in irreversible neuronal necrosis (Allen and Iversen, Science, 1990). Both competitive and non-competitive NMDA antagonists cause such effects. Here we report that certain anti-cholinergic compounds, when administered systemically, effectively protect cingulate/retrosplenial neurons against the neurotoxic action of the powerful NMDA antagonist, MK-801. Although all of the effective anti-cholinergic compounds have significant affinity for both M1 and M2 muscarinic receptors, their order of potencies in preventing MK-801 neurotoxicity (scopolamine > benztropine > trihexyphenidyl = atropine > biperiden > procyclidine > benactyzine > diphenhydramine) correlates better with their order of binding affinities for the M1 than M2 receptor. Using the chick embryo retina, we determined that high concentrations of these anticholinergic compounds do not interfere with the ability of MK-801 to protect retinal neurons against NMDA toxicity. These compounds, therefore, provide a simple and safe method for eliminating a potentially serious side effect associated with the use of NMDA antagonists as neuro-protective drugs.

461.7

DOMOIC ACID: A DEMENTIA-INDUCING EXCITOTOXIC FOOD POISON WITH KAINIC ACID RECEPTOR SPECIFICITY G.R. Stewart, C.F. Zorumski, M.T. Price, and J.W. Olney. Dept. of Psychiatry, Washington University Sch. of Med., St. Louis, MO 63110.

Domoic acid (Dom), a structural analog of the excitotoxic amino acid, glutamate (Glu), is believed to be the mussel neurotoxin responsible for a food poisoning incident in Canada in 1987 that killed some individuals and left others cognitively impaired. Since information pertaining to Dom excitotoxicity is limited, we have evaluated the neuroexcitatory properties of Dom *in vitro* (cultured hippocampal neurons) and its neurotoxic properties both *in vitro* (chick embryo retina) and *in vivo* (adult rat).

In vitro, the properties of Dom were compared with those of kainic acid (KA), *N*-methyl-D-aspartate (NMDA), and Quisqualate (Quis), each of which is a prototypic agonist at a different subtype of Glu receptor. Under voltage clamped conditions, currents induced in hippocampal neurons by Dom were identical to currents induced by KA; both displayed a linear current/voltage relationship (in contrast to NMDA currents) and were non-desensitizing (in contrast to Quis currents). Dom currents were not blocked by NMDA antagonists but were blocked by CNQX, an antagonist of non-NMDA receptors. In the chick embryo retina, Dom induced the same distinctive lesion pattern as KA which differed from the NMDA or Quis lesion, and the Dom lesion was blocked by CNQX but not by NMDA antagonists. Subcutaneous administration of Dom (2.5-3 mg/kg) to adult rats resulted in an acute seizure-brain damage syndrome similar to that caused by systemic KA (12 mg/kg), and analogous to the neurotoxic syndrome observed in the human food poison victims.

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461.8

EXOGENOUS EXCITATORY AMINO ACID NEUROTOXICITY IN VITRO CAN BE INDUCED BY CYCLIC AMP. A. Morandi*, L. Facci, D. Milani*, A. Leon and S.D. Skaper. Fidia Research Laboratories, 35031 Abano Terme (PD), Italy.

Glutamate, an excitatory amino acid (EAA) that exerts a trophic effect on immature cerebellar granule cells in culture, is toxic at later stages of cellular maturation. We observed that some granule cell preparations failed to develop this sensitivity to excitotoxin injury. As a first approach to understanding the nature of this resistance, prolonged pretreatment (18h) with db-cAMP or forskolin (FSK) was found to confer, in a dose dependent way, sensitivity to the neurotoxic actions of glutamate, aspartate and kainate. Shorter treatment times, or addition of db-cAMP only during EAA exposure or afterwards failed to be effective. The neurotoxic action of glutamate in these sensitized cells was blocked by Mg^{2+}, ganglioside GM1, and inhibitors of protein synthesis. Following removal of db-cAMP, the granule cells again became resistant to glutamate neurotoxicity after 48 h. These observations may provide new insights into the mechanisms of excitotoxicity and their possible role in human CNS pathologies.

461.9

AMINO ACID NEUROTOXICITY IN CORTICAL SLICES.
H.C.Sullivan and A.R.Kriegstein Dept.of Neurology, Stanford Univ. Med. Center, Stanford, CA 94305.

There is evidence that glutamate neurotoxicity mediates hypoxic injury in select neuronal populations. Studies have shown that dissociated cell cultures of turtle neurons are relatively resistant to glutamate neurotoxicity (Wilson and Kriegstein, Soc.Neurosci.Abst. 15:763.) Because turtle brain is resistant to prolonged periods of anoxia, we have been able to measure delayed neuronal degeneration in adult turtle cortical slices 24 hours after exposure to excitatory amino acids. Cortical slices (400 um) from adult diving turtles were exposed to glutamate (Glu), NMDA, and kainate (KA) for 30 minutes, and incubated for 24 hours. Measurement of LDH released into the bathing media correlated with morphological injury. The LD_{50} values from the dose-toxicity curves for Glu, NMDA, and KA were 2.2mM, 18uM, and 0.3uM respectively. The Glu uptake inhibitor, dihydroKA, reduced the LD_{50} for Glu to 100uM, but threo-OH-aspartate blocked Glu toxicity. APV and CNQX were effective antagonists for NMDA and KA neurotoxicity. We conclude that the slice method is a rapid and reliable means of assessing EAA neurotoxicity in turtle cortex.

461.10

SENSITIVITY TO AMPA INDUCED BRAIN INJURY TRANSIENTLY PEAKS EARLY IN POSTNATAL DEVELOPMENT, William H. Trescher, John W. McDonald, Michael V. Johnston, The Kennedy Research Institute and The Johns Hopkins University School of Medicine, Department of Neurology, Baltimore, MD 21205.

The ontogeny of quisqualate subtype excitatory amino acid (EAA) receptor mediated neurotoxicity was studied in rats at 8 ages between postnatal day (PND) 1-90 (adult). Animals received unilateral, anterior striatial, stereotaxic injections of either alpha-amino-3-hydroxy-5-methyl-4-isoxazoleacetic acid (AMPA) (25 nmol/0.5 ul) or quisqualatic acid (QA) (100 nmol/0.5 ul). Severity of brain damage was evaluated by comparing regional cross-sectional areas of injected and contralateral cerebral hemispheres of brains 5 days after excitotoxin injection. The area of the ipsilateral corpus striatum was reduced by 25% at PND 1, which was equivalent to the severity of damage in adults; neuronal necrosis was limited to the injection tract. Between PND 5 and 28, the extent of AMPA induced neurotoxicity exceeded that in adults, transiently peaking at PND 10, when the damage to the striatum was 4-fold greater than in adults. From PND 5 to 10, the excitotoxic reaction to AMPA spread to progressively larger areas of the ipsilateral hemisphere, and involved medial aspects of the contralateral hemisphere at PND 7 and 10. In AMPA injected animals, ipsilateral cortical cross-sectional area was reduced by 11% in adults. In contrast, at PND 10 there was extensive confluent neuronal necrosis to the cerebral cortex, and the extent of damage exceeded adult values by a factor of 8. After PND 10, the extent of AMPA induced damage gradually decreased in all regions. A similar developmental pattern of sensitivity to QA toxicity was observed with maximal damage to the striatum at PND 10. The data demonstrate that EAA induced neurotoxicity mediated by the QA receptor subtype is enhanced in the developing rat brain with a pattern that is distinct from the other EAA receptor subtypes.

461.11

HYPERBILIRUBINEMIA IS ASSOCIATED WITH ENHANCED SUSCEPTIBILITY TO EXCITOTOXIC BRAIN INJURY. John W. McDonald, Steven M. Shapiro+*, Faye S. Silverstein@ and Michael V. Johnston, Departments of Neurology and Pediatrics, Johns Hopkins University and Kennedy Institute, Baltimore, MD; +Medical College of Virginia, Richmond, VA; and @University of Michigan, Ann Arbor, MI.

The pathophysiology of bilirubin encephalopathy is not well defined. The Gunn rat, which has an inherited deficiency of glucuronyl transferase, is commonly used as a model of human kernicterus. Homozygous (jj) animals have an unconjugated hyperbilirubinemia, and manifest a pattern of brain injury that resembles human bilirubin encephalopathy. Heterozygous (Nj) animals have 50% glucuronyl transferse activity, are not jaundiced, and do not develop signs of bilirubin encephalopathy. To examine the hypothesis that hyperbilirubinemia increases the susceptibility to excitotoxic brain injury, the sensitivity of PND 7 Nj and jj Gunn rats to brain injury induced by the EAA analogue NMDA was compared. In experiment #1, PND 7 littermates received a unilateral intrastriatal stereotaxic injection of 5 nmol NMDA (n= 4 jj, 4 Nj). In experiment #2, 5 jj and Nj littermate pairs received an i.p. injection of 200 mg/kg sulfamethoxazole (SM) 4 hrs prior to unilateral intrastriatal injection of NMDA. The severity of brain injury was quantitated 5 days later by comparison of regional cross-sectional areas in injected and contralateral cerebral hemispheres. NMDA mediated brain injury was enhanced in jj animals compared to Nj littermates (%Damage=%reduction of cross-sectional area in injected compared to non-injected hemisphere, mean±SEM: Striatum, -15.5±3.5% Nj vs -24.1±2.4% jj, $p<0.05$; hippocampus, -8.6±5.1% Nj vs -19.5±6% jj, p=ns). Pretreatment with sulfamethoxazole, which elevates brain tissue bilirubin, markedly increased the severity of NMDA induced brain injury in jj, but not Nj, rats (%Damage, mean±SEM: striatum, -13.8±1.3% Nj vs -34.4±2.5% jj, $p<0.001$; hippocampus, -4.5±9.2% Nj vs -40.0±9.0% jj, $p<0.05$). The results indicate that hyperbilirubinemia in Gunn rats is associated with an increased susceptibility to excitotoxic brain injury.

461.12

ACTION OF OXYGEN FREE RADICALS ON THE REDOX MODULATORY SITE OF THE NMDA RECEPTOR.
E. Aizenman, K.A. Hartnett*, & I.J. Reynolds, Depts. Physiology and Pharmacology, U. of Pittsburgh Sch. of Med., Pittsburgh., PA 15261.

NMDA responses in central neurons can be modified by sulfhydryl redox reagents (Neuron, 2: 1257; 1989). This led us to search for endogenous agents acting at the NMDA receptor redox modulatory site. Fura-2-measured rises in Ca^{2+} produced by 30 μM NMDA + 1 μM glycine in cultured rat cortical neurons were enhanced by 0.3-10 mM of the reductant DTT. Treatment of neurons with the oxidizing agent DTNB (0.5 mM), or with the oxygen free radical generating system of xanthine (X; 1 mM)/xanthine oxidase (XO; 0.05 U/ml), could reverse the effect of DTT and diminish the native response to NMDA & glycine. The actions of X/XO (or DTNB) were further reversed by DTT. It was also observed that the 0.5 mM DTT-induced enhancement of NMDA (50 μM)-mediated toxicity in cortical cultures was always reversed by X/XO (100 μM/0.005 u/ml). These results show that NMDA responses can be modified by an endogenous oxygen free radical generating system acting via the redox modulatory site on the NMDA receptor.

MOLECULAR NEUROBIOLOGY OF 5HT RECEPTORS

462.1

ISOLATION OF A DROSOPHILA SEROTONIN RECEPTOR: AN UNUSUAL MEMBER OF THE G PROTEIN-COUPLED RECEPTOR FAMILY.
Hen R.*, Witz P.*, Maroteaux L.*, Gombos G.#, Borrelli E.* and Amlaiky N.* *LGME/CNRS-U184/INSERM, URA DO589 CNRS, #Centre de Neurochimie, Strasbourg, France.

A Drosophila serotonin receptor cDNA was isolated employing low stringency hybridization with oligonucleotides corresponding to consensus sequences present in G protein-coupled receptor. The deduced protein (5HT-dro receptor), which contains 564 amino acids, exhibits homology to the human 5HT1A receptor. Unlike all other G protein-coupled receptors which have a predicted seven transmembrane domain structure, the hydropathy profile of the 5HT-dro receptor reveals eight putative transmembrane domains. This receptor contains also a 20 amino acid long glycine-serine repeat, which is a potential glycosaminoglycan attachment site. Such repeats are found in certain proteoglycans and in the Drosophila clock protein period. Probes derived from this receptor hybridize to a single mRNA species 5 kbp in length, which is found predominantly in adult Drosophila heads. In order to characterize this receptor we expressed it in NIH 3T3 cells. In these transfected cells serotonin induced an increase in cAMP levels that was concentration dependent and saturable. The half maximum stimulation was obtained with 10^{-7} M serotonin, and stimulation was inhibited by ergot alkaloïds such as dihydroergocryptine.

462.2

COUPLING OF VACCINIA VIRUS EXPRESSED 5-HT1A RECEPTORS TO AN ENDOGENOUS K^+ CHANNEL IN RAT ATRIAL CELLS. A. Karschin, B.Y. Ho*, C. Labarca*, O. Elroy-Stein*, B. Moss*, N. Davidson, and H.A. Lester. Caltech, Division of Biology 156-29, Pasadena, CA 91125, and LVD, NIH, Bethesda, MD 20892.

Recombinant vaccinia viruses (VV) represent unique vectors for heterologous gene expression in eukaryotic cells. We constructed a recombinant VV harboring the human 5-HT1AR and expressed it in primary cultures of neonatal rat atrial myocytes. For maximum expression a novel strategy was used based on coinfection with one virus carrying the 5-HT1AR gene downstream of a bacteriophage T7 promoter and an encephalomyocarditis virus sequence, and a second VV containing the T7 polymerase gene under control of a VV promoter (Elroy-Stein et al., PNAS 86:6126, 1989). The 5-HT1AR subtype seems to couple directly to K^+ channels via G proteins in the hippocampus. A similar pathway is known for ACh in atrial cells, where muscarinic AChRs activate an inwardly rectifying K^+ current (I_{ACh}). We used whole-cell recordings of atrial cells to study whether a VV-expressed 5-HT1AR would functionally couple to an intrinsic K^+ conductance. In cells that were either non-infected or infected with wild-type virus, I_{ACh} was induced by ACh (10 μM), but not by 5-HT (10 μM). Only after the coinfection with the two recombinant viruses, 5-HT activated an inwardly rectifying K^+ current in atrial cells. 5-HT responses were observed as early as 15 h post-infection and could be mimicked by the selective 5-HT1AR agonist 8-OH DPAT. The expressed 5-HT1AR is suggested to couple to a pre-existing muscarinic excitation pathway in cardiac atria. Support: Max-Kade- and Del Webb Foundation, GM-29836, GM-10991 (NIH).

462.3

INTRINSIC ACTIVITY OF TETRAHYDROPYRIDINYL INDOLES AT 5-HT_{1A} RECEPTORS NEGATIVELY COUPLED TO ADENYLATE CYCLASE. L.J.Cornfield, G.Lambert*, T.Dahlgren[1*], S.S.Nikam[1*], Y.Yang[1*], A.R. Martin[1*] & D.L.Nelson, Dept. of Pharmacology & Toxicology and [1]Dept. of Pharmaceutical Sciences, College of Pharmacy, University of Arizona, Tucson, AZ 85721 USA.

This study extends work previously undertaken to determine optimal 5-HT_{1A} affinity and selectivity, using conformationally rigid analogs of 5-HT itself (Taylor et al., Mol. Pharmacol. 34:42, 1988). The series consisted of analogs of 3-(1,2,5,6-tetrahydropyridin-3-yl)indoles (3-THPI) and 3-(1,2,5,6-tetrahydropyridin-4-yl)indoles (4-THPI). Most analogs produced complex inhibition curves in the forskolin-stimulated adenylate cyclase (FSC) assay (DeVivo & Maayani, J. Pharmacol. Exp. Ther. 238:248, 1986) using male Sprague-Dawley rat hippocampus. These inhibition curves contained a pindolol-sensitive component, suggesting at least partial 5-HT_{1A} agonist activity. Analogs producing only weak inhibition of FSC were able to reverse 5-HT-induced inhibition by varying degrees. The 4-THPI analogs generally had higher 5-HT_{1A} binding affinities than the 3-THPI analogs. Within each group of analogs, it appeared that the higher the 5-HT_{1A} affinity, the greater the 5-HT_{1A} agonistic activity. Further analogs must be tested to confirm this trend. Analogs with a carboxamido group in the 5-indole position did not follow these trends.(Sup.by NS16605 and NS01009).

462.4

PRE- AND POSTSYNAPTIC 5-HT_{1A} RECEPTORS EXHIBIT DIFFERENT ELECTROPHYSIOLOGICAL PROPERTIES: I - EFFECTS OF SPIPERONE. C. de Montigny, A. Lista and P. Blier. Neurobiological Psychiatry Unit, Dept. of Psychiatry, McGill University, Montreal, Quebec, Canada H3A 1A1.

Single-cell extracellular recordings were obtained from dorsal raphe 5-HT neurons and from dorsal hippocampus CA_3 pyramidal neurons in chloral hydrate-anesthetized rats.

The effect of microiontophoretic applications of 5-HT and of 8-OH-DPAT, but not that of GABA, onto dorsal raphe 5-HT neurons was readily antagonized by acute spiperone (1 mg/kg, i.v.). The same dose of spiperone did not alter the response of pyramidal neurons of the dorsal hippocampus either to microiontophoretically-applied 5-HT or to endogenous 5-HT released by the electrical stimulation of the ascending 5-HT pathway.

However, the response of dorsal hippocampus pyramidal neurons to both endogenous and exogenous 5-HT was markedly attenuated 24 hr after the i.p. administration of 5 mg/kg of spiperone. Such an inactivation of postsynaptic 5-HT_{1A} receptor response could not be detected 12 hr after the i.p. administration of 5 mg/kg of spiperone, nor 24 hr after lower doses of spiperone (1 and 2.5 mg/kg, i.p.). Neither ketanserine (5 mg/kg, i.p.), a 5-HT_2 antagonist, nor haloperidol (5 mg/kg, i.p.), a D_2 antagonist, modified dorsal hippocampus pyramidal neurons response to either exogenous or endogenous 5-HT, 24 hr after their administration, indicating that the effect of spiperone is attributable to its 5-HT_{1A} affinity rather than its 5-HT_2 or D_2 affinities.

These results show that spiperone acts as a classical antagonist at the somatodendritic 5-HT_{1A} autoreceptor in the dorsal raphe, but not at postsynaptic 5-HT_{1A} receptors in the dorsal hippocampus. The mechanism whereby a single dose of the 5-HT_{1A} antagonist spiperone induces a delayed inactivation of the latter receptors remains to be unveiled.

462.5

PRE- AND POSTSYNAPTIC 5-HT_{1A} RECEPTORS EXHIBIT DIFFERENT ELECTROPHYSIOLOGICAL PROPERTIES: II- EFFECTS OF PERTUSSIS AND CHOLERA TOXINS. P. Blier, A. Lista and C. de Montigny. Neurobiological Psychiatry Unit, Department of Psychiatry, McGill University, Montreal, Quebec, Canada H3A 1A1.

5-HT_{1A} autoreceptors located on the soma of 5-HT neurons and postsynaptic 5-HT_{1A} receptors on the cell body of CA, hippocampus pyramidal neurons are coupled to G_i proteins. In the present experiments, the effect of microiontophoretic application of 5-HT onto dorsal raphe 5-HT neurons and onto dorsal hippocampus CA_3 pyramidal neurons was assessed 1 to 2 weeks following in situ injections of pertussis or cholera toxin (1 µg in 2 µL of saline). In addition, the response of the same postsynaptic neurons to endogenous 5-HT released by the electrical stimulation of the ascending 5-HT pathway was assessed in the same rats, as 5-HT terminals make synaptic contacts on the dendrites of these neurons.

Pertussis toxin, which inactivates G_i proteins, markedly attenuated the response of 5-HT neurons and that of CA_3 neurons to the microiontophoretic application of 5-HT, but left unaltered the effectiveness of the stimulation of the 5-HT pathway in suppressing the firing activity of the latter neurons. In contrast, cholera toxin, which interferes with G_s-coupled mechanisms, did not modify the responsiveness of 5-HT neurons to microiontophoretically-applied 5-HT, but reduced both the effectiveness of the stimulation of the 5-HT pathway and that of the microiontophoretic applications of 5-HT on CA_3 neurons.

These results suggest that: 1) the 5-HT_{1A} autoreceptor of 5-HT neurons is coupled to a G_i, but not to a G_s protein; 2) there may exist on CA_3 pyramidal neurons intra-synaptic 5-HT_{1A} receptors on their dendrites which are coupled to a G_s but not to a G_i protein, and extra-synaptic 5-HT_{1A} receptors on their cell body which are coupled to both G_s and G_i proteins.

462.6

DEVELOPMENTAL REGULATION OF SEROTONIN RECEPTOR (5HT2/5HT1C) AND mRNA LEVELS. Bryan L. Roth, Mark W. Hamblin, Roland D. Ciaranello, Lab Dev Neurochem, Department of Psychiatry, Stanford Univ Med School, Stanford, CA 94305.

Serotonin (5-hydroxytryptamine; 5T) receptors have been shown to be regulated by a diverse array of factors, some of which appear to be developmentally regulated. Two of these receptors (5HT2 and 5HT1c) have been cloned and implicated as mitogens. We have discovered that each receptor appears to have a distinct pattern of receptor and mRNA expression during rat perinatal brain development. The 5HT2 receptor, for instance, increases 8-fold during ontogeny while its corresponding mRNA increases approximately 13-fold (both $p<0.001$); levels of receptor gene expression were unaffected by receptor antagonist (mianserin) treatment during the perinatal or adult periods. The 5HT1c receptor gene appeared to have a number of discrete "bursts" of expression depending upon the brain area and the developmental period. Thus, for instance, a pulse of receptor gene expression occured in the areas of the reticular and raphe nuclei during the second post-natal week while choroid plexus gene expression was evident at the earliest time point studied as assessed by *in situ* hybridization (embryonic day 17). These results imply the existence of distinct developmental "switches" in various brain regions responsible for controlling the expression of different serotonin receptor genes.

462.7

NEONATAL 5,7-DHT LESIONS ALTER [^{3}H]MESULERGINE-LABELLED 5-HT1C RECEPTORS IN RAT BRAIN. M. R. Pranzatelli, Columbia Univ., New York, NY 10032.

5-HT receptor denervation supersensitivity has been proposed to explain behavioral supersensitivity to L-5-HTP in rats with 5,7-dihydroxytryptamine (5,7-DHT) lesions. No upregulation of 5-HT2 binding sites has been found despite supersensitivity to putative 5-HT2,1C drugs. To test the hypothesis that the 5-HT1C properties of these drugs are involved instead, we measured 5-HT1C receptors in rats one month after making neonatal 5,7-DHT lesions by intraperitoneal injection. Sites labelled with [^{3}H]mesulergine (with 10 µM 5-HT as displacer) showed a distinct regional distribution: frontal cortex>> hippocampus>brainstem>diencephalon>>cerebellum. In the presence of 20 nM spiperone to block 5-HT2 sites, 31%, 70%, 65%, 70%, and 73% of the sites remained, respectively. 5,7-DHT lesions upregulated these sites in frontal cortex (+29%) and hippocampus (+25%). There were no changes in Bmax in other regions. Kd and nH were unchanged. Changes in Bmax paralleled 5-HT concentrations measured by HPLC. These data suggest that 5-HT1C rather than 5-HT2 receptor denervation supersensitivity may explain enhanced behavioral responses to 5-HT2,1C drugs in rats with 5,7-DHT lesions. (Supported by NIH grant 1-KO8-NS01158 (CIDA), the Myoclonus Research Fund, the United Cerebral Palsy Education and Research Foundation (R381-88), and the William Randolph Hearst Foundation).

462.8

ISOLATION, PURIFICATION TO HOMOGENEITY AND SEQUENCE OF A BRAIN ENDOCOID FOR ^{3}KETANSERIN RECOGNITION SITES (KBI). J.A. Apud*, A. Guidotti, Y. Ito*§, B. Martin*†, M.L. Barbaccia and E. Costa. FGIN, Georgetown University, Washington, D.C. 20007; §Laboratory of Biophysical Chemistry, NHLBI and †Laboratory of Molecular Neurogenetics, NIMH, Bethesda, MD 20892.

A peptide that selectively displaces ^{3}H-KET binding from crude synaptic membranes has been recently extracted, purified to homogeneity and sequenced. Major steps for its purification include: a)homogenization (1:6,w:v) of rat brain with 0.1M CH_3COOH and heat inactivation (90°C, 10 min), b) filtration of the 40,000xg supernatant on Biogel P-10 column equilibrated with 0.1M CH_3COOH:20% CH_3OH, c) precipitation in 60% ammonium sulphate, d) countercurrent chromatography (CCC) of the 40,000 xg supernatant, e)C_{18} µBondapak reverse phase HPLC, f) C_{18} PTH reverse phase HPLC. A partial sequence containing 17 amino acids has been identified using the Edman degradation technique. The hydropathy profile of the partial sequence is in agreement with the physico-chemical behavior of the endocoid revealed both in the CCC and HPLC purification steps. KBI is pronase-sensitive and fails to displace ^{3}H-mianserin and ^{3}H-imipramine at concentrations which inhibit 60% of ^{3}H-KET specific binding. The activity of the KBI on platelet aggregation and on phosphoinositide hydrolysis in rat cerebral cortex indicate that KBI interact with the 5-HT 2 receptor domain.

462.9

AFFINITIES OF THE TWO ENANTIOMERS OF FLUOXETINE FOR SUBTYPES OF SEROTONIN (5-HYDROXYTRYPTAMINE, 5HT) RECEPTORS. D.T. Wong, P.G. Threlkeld*, R.D. Marsh*, F.P. Bymaster*, L.R. Reid* and D.W. Robertson*. Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN 46285.

The two enantiomers of fluoxetine exhibit similar pharmacology as 5HT uptake inhibitors, with eudismic ratios near unity (Wong et al, Drug Dev.Res.6:397, 1985). The R and S enantiomers and racemic fluoxetine inhibited 5HT uptake in human platelets at 50% 3.6, 3.9 and 4.6 nM (IC_{50}), respectively, but have relatively weak affinity for 5HT-1 (A,B,D) and 5HT-2 receptors, as indicated by the micromolar IC_{50} concentrations to inhibit binding of radioligands to these receptors. However, the R enantiomer and racemic fluoxetine inhibited ^{3}H-mesulergine binding to 5HT-1C receptors in bovine choroid plexus, with IC_{50} values of 200 and 450 nM, respectively, while the IC_{50} of S-fluoxetine was 17,000 nM. Fluoxetine and the two enantiomers inhibited ^{3}H-LY278584 binding to 5HT-3 receptors, with IC_{50} values at micromolar concentrations. These concentrations were substantially higher than the IC_{50} of 10 nM for fluoxetine to inhibit ^{3}H-quipazine binding to its recognition sites (Schmidt & Peroutka, Eur.J.Pharmacol. 163:397, 1989). Thus, R-fluoxetine, besides being a potent 5HT uptake inhibitor, has an intermediate affinity for 5HT-1C receptors. The effects of racemic fluoxetine appear to be mediated primarily via inhibition of 5HT uptake, but further studies should determine whether occupancy of 5HT-1C receptors is reflected in any pharmacological effects.

462.10

LOCALIZATION OF CENTRAL 5-HT3 RECEPTORS IN THE RAT USING ^{3}H-ZACOPRIDE AND ^{125}I-ZACOPRIDE. L.E. Schechter, F. Bolanos*, A.M. Laporte* M. Hamon*and H. Gozlan*. INSERM U. 288, CHU Pitié-Salpêtrière, 91 Bld. de l'Hôpital, 75634 Paris Cedex 13, France

To date, various lines of evidence have suggested that central 5-HT3 binding sites are involved in anxiety, depression and schizophrenia as well as emesis and pain-modulation. In order to determine the possible loci of action for these effects, the distribution of 5-HT3 binding sites was examined in the rat brain and spinal cord using both membrane homogenates and autoradiography with the tritiated and iodinated derivatives of the potent and selective 5-HT3 antagonist, zacopride. 5-HT3 binding sites were notably found in the nucleus of the solitary tract and the dorsal nucleus of the vagus nerve, i.e. structures perhaps involved in the emetic reflex. High densities of 5-HT3 binding sites were also observed within the spinal trigeminal nucleus and the superficial layers of the dorsal horn of the spinal cord, where 5-HT3 ligands probably act as pain-modulating drugs. Finally, the amygdala (cortical and basolateral nuclei), hippocampus and entorhinal cortex also contained 5-HT3 binding sites, which probably mediate the behavioral effects of 5-HT3 agents. Further investigations in rats treated by 5,7-dihydroxytryptamine, kainic acid or capsaicin indicated that 5-HT3 binding sites are located on postsynaptic targets in limbic areas, and (partly) on primary afferent fibers in the spinal cord.

462.11

RESPONSES OF ENTERIC NEURONS TO ANTI-IDIOTYPIC ANTIBODIES THAT RECOGNIZE 5-HT_3 AND 5-HT_{1P} RECEPTOR SUBTYPES P. R. Wade*, H. Tamir, and M. D. Gershon, Departments of Anatomy and Cell Biology , Columbia Univ. P & S and N.Y. State Psych. Inst. New York, NY.

Two 5-HT receptor subtypes, 5-HT_{1P} and 5-HT_3, have excitatory effects on enteric neurons. The 5-HT_{1P} receptor mediates a long lasting depolarization associated with an increase in input resistance. The 5-HT_3 receptor mediates a transient depolarization during which the input resistance decreases. Polyclonal anti-idiotypic antibodies (anti-id Aß) have been raised by immunizing rabbits with affinity purified antibodies to 5-HT. The resulting crude anti-id Aß were purified by affinity chromatography and shown by immunocytochemistry to bind to a subset of enteric neurons. Anti-id Aß were applied by microejection to guinea pig myenteric neurons and found to mimic both 5-HT_3- and 5-HT_{1P}-mediated responses to 5-HT. Responses to the anti-id Aß were antagonized by desensitization of 5-HT receptors and by renzapride (BRL 24924), which antagonizes responses to 5-HT mediated either by 5-HT_3 or 5-HT_{1P} receptors. Following application of anti-id Aß, both 5-HT_3 and 5-HT_{1P} responses to 5-HT were inhibited (5-HT_{1P} > 5-HT_3). This inhibition appeared to be specific because the anti-id Aß did not affect responses to carbachol or substance P. It is concluded that the anti-id Aß binds to 5-HT receptors. Its affinity appears to be greater for 5-HT_{1P} than for 5-HT_3 receptors. Supported by grants NS 12969, MH 37575 and the PMAF.

PROCESS OUTGROWTH, GROWTH CONES AND GUIDANCE MECHANISMS X

463.1

BEHAVIOR OF LIVE RETINAL AXON GROWTH CONES IN THE OPTIC CHIASM P. Godement* and C.A. Mason, *Inst. Neurosciences, CNRS, Univ. P.M. Curie, Paris 75005, and Dept. Pathology, Coll. Physicians and Surgeons, Columbia University, New York, N.Y. 10032.

Previous studies on a variety of neural systems have demonstrated that growth cone morphology is position-specific, becoming more complex in regions where fibers change direction or sort out among themselves. Our analysis of DiI-labeled axons in the mouse optic chiasm (Godement et al., Neuron, in press) show that within this decision region, the most complex growth cones develop on uncrossed axons as they turn sharply toward the ipsilateral optic tract. To link morphology with behavior, we observed growth cones extending in real time. A semi-intact preparation including retinae, optic nerves and a slab of ventral diencephalon, was dissected from mouse embryos at E14-16, small injections of DiI were made in the retina, and the preparation kept in culture for two days. Growth cone behavior was monitored over several hours using low levels of incident fluorescent illumination and time-lapse video recording. Many growth cones advance steadily at rates of 15-50 microns/hr, while others stall, retracting and regrowing repeatedly. Growth cones rapidly change shape, becoming longer with fewer filopodia when advancing, and spread with more filopodia, when resting. Retraction and regrowth is often associated with slight shifts in directionality of the growth cone. Thus, growth of retinal fibers in their natural environment is a dynamic process, involving changes in form, saltatory growth, and process retraction. This analysis should indicate the role of such growth cone behaviors within pathways of straight growth and in the selection of appropriate trajectories within decision regions. (Supported by NS 27615).

463.2

RETINAL AXON NAVIGATION IN THE MOUSE OPTIC CHIASM: MIDLINE CUES AND CELL-CELL RELATIONS. C.A. Mason, J.P. Misson*, R. Blazeski, and P. Godement**, Dept. Pathology, Coll. Physicians and Surgeons, Columbia University, New York, N.Y., *Dept. Pediatrics, C.H.U. Sart-Tilman, Liege 4000, Belgium, and **Inst. Neurosciences, CNRS, Univ. P. M. Curie, Paris 75005.

As mouse retinal axons navigate through the optic chiasm, axons projecting to the contralateral side of the brain cross through the chiasm midline. Uncrossed axons initally travel with crossed fibers, but turn back abruptly at the border of a 100 micron-wide zone along the midline (Godement et al., Neuron, in press). As uncrossed fibers turn, their growth cones become highly complex. To examine the cellular structure of the chiasm midline, we immunostained the chiasm during the period of axon growth (E14-17) with a number of glial and neuronal cell-surface molecules. Antibodies to RC2, an antigen on immature radial glia in mouse CNS (Misson et al., *Dev. Br. Res.* 44:95, 1988) stained a palisade of radial fibers extending from the floor of the third ventricle through the chiasm to the pia,100-200 microns on either side of the midline. In the lateral chiasm, small cells with short processes were also stained. To understand the cell-cell relations of retinal growth cones in the chiasm, we examined identified DiI-labeled growth cones that were photoxidized and processed for EM. Growth cones on straight-growing fibers fasciculated on bundles of other axons. The complex growth cones on turning fibers contacted multiple processes and abutted cell bodies of unidentified cells. Clear and coated vesicles were prominent in the turning growth cones and in the profiles they contacted. This analysis should aid in identifying the cues for crossed and uncrossed fiber navigation, and may implicate properties intrinsic to each of these fiber populations that underly the differential response to these cues. (Supported by NS 27615).

463.3

AXON NAVIGATION AT THE MAMMALIAN OPTIC CHIASM: DIRECT OBSERVATION USING FLUORESCENT TIME-LAPSE VIDEO MICROSCOPY. D.W. Sretavan. Laboratory of Neurobiology, Rockefeller University, NY 10021.

Developing mammalian retinal ganglion cell axons make highly specific pathway choices at the optic chiasm (Sretavan, D.W. Soc. Neurosci. Abstr., 15: 960, 1989). To better understand axon pathfinding in this region, ganglion cell axons were directly observed growing into the optic chiasm in an intact preparation of the embryonic CNS. To do so, the retinas, optic nerves together with the chiasm and tracts were isolated from E13-E18 mouse embryos. DiI was deposited onto the retina to label ganglion cell axons and preparations maintained at 37°C on an inverted microscope. Time lapse images of axons were obtained every 30-60 seconds up to 12 hours using a CCD camera.

Several axon turning behaviors were seen at the optic chiasm. Retinal axons entering the ipsilateral optic tract often did so by making gradual 90° turns while maintaining continuous growth cone activity. On the other hand, some axons avoided entering the ipsilateral optic tract by retracting the distal 20 μm of its axon and simultaneously extending a new process in a different direction. Episodes where axons belonging to the two eyes encountered each other often resulted in a temporary pause in growth followed by the veering away of axons in a new direction upon recovery. This behavior was seen only in a subset of axon-axon encounters, suggesting that these repulsive interactions occur between axons from specific regions of the two retinas.

Unlike conventional histological methods, this preparation allows the examination of dynamic interactions between retinal axons and axon navigation within the native enivronment of the developing CNS. In addition. it potentially allows the direct perturbation of specific interactions between retinal axons and their environment and thus may help in furthering our understanding of axon guidance mechanisms.

I thank Torsten Wiesel for encouragement and continued support, and Larry Katz and his laboratory for expert advice on video microscopy and the use of equipment.

463.4

DYNAMIC BRANCHING PATTERNS IN OPTIC FIBER TERMINAL ARBORS WITHIN THE TECTUM N. A. O'Rourke and S. E. Fraser. Dept. of Physiol. & Biophysics, University of CA, Irvine, CA 92717.

Retinal ganglion cell axons project into the optic tectum where they branch to form terminal arbors, which are arranged in a topographic pattern. *In vivo* observations have revealed the dynamic arborization patterns of these fibers during the initial formation of the retinotopic map. The optic fibers were labeled with a fluorescent vital dye and visualized within the tecta of live *Xenopus laevis* tadpoles using a laser-scanning confocal microscope to resolve the complex three-dimensional structure of the arbors. Observation of the labeled fibers over a period of days has revealed that all the terminal arbors, even those with stable dimensions, were continuously remodeled through branch extension and retraction. To better characterize the branch dynamics, the arbors were visualized at shorter time intervals. Branches formed either through bifurcation at an axon tip or, more commonly, at more proximal positions along the length of another branch. Two classes of branches were found in the arbors. Short branches of less than 4 microns in length, termed "spikes", exhibited a rapid rate of remodeling and were seldom present for more than an hour. Longer branches showed much slower rates of extension and retraction which varied somewhat from one arbor to the next. Interestingly, the branch growth within an arbor was often restricted to one or two branches at any one time, suggesting that the growth machinery within the axon can be shunted selectively to one area of an arbor during the remodeling process. Because all the arbors were dynamic, even those with stable dimensions and tectal positions, these findings suggest that continual remodeling of arbors may be a universal feature of neuronal projections, even in systems previously thought to be static. (NIH EY08363).

463.5

AVOIDANCE RESPONSE AND COLLAPSE OF FISH TEMPORAL RETINAL GROWTH CONES UPON CONTACT WITH CAUDAL TECTAL MEMBRANES OF EMBRYONIC CHICK

C.A.O. Stuermer, M. Bastmeyer, J. Vielmetter,
Friedrich-Miescher-Lab./Max-Planck-Gesellschaft, Tübingen, FRG

Caudal tectal membranes of embryonic chick (Walter et al., 1987) and caudal tectal membranes of adult goldfish (Vielmetter et al., 1990) contain a repellent guiding component for temporal retinal axons. Temporal retinal axons of fish respond to both, the repellent component of fish and to that of E9 chick. The chick component, however, exerts a stronger repellent influence on fish temporal axons than that of fish.

We observed the avoidance behavior of fish growth cones upon contact with chick caudal tectal membranes in a modified in vitro stripe assay with time lapse videomicroscopy.

Axon elongate on 90 μm wide laminin lanes bounded by caudal membranes. Upon contact with caudal membranes on one side growth cones sent up to 20 μm long filopodia and lamellipodia onto the membranes, but retracted them rapidly while the growth cone continued to grow on the laminin lane. At the end of the laminin lane, filopodia and lamellipodia contacted membranes on 3 sides. Here, the growth cones collapsed and the axons retracted leaving thin strands of axon material behind. In control experiments, using rostral instead of caudal membranes, fish temporal axons grew freely into the membranes. The growth cone avoidance behavior will be demonstrated in a time lapse videosequence.

463.6

INTERACTIONS BETWEEN MOTOR NEURON DENDRITES AND DORSAL ROOT AFFERENT AXONS IN DEVELOPING MAMMALIAN SPINAL CORD. W.D. Snider, N. Gorukanti and C. Tsering, Dept. of Neurology, Washington Univ. Med. School, St. Louis, Mo. 63110

We have used two chromophores DiI and DiA (Di-16-ASP) in fixed tissue to study interactions between growing dendrites of spinal motor neurons and Ia afferent axons of dorsal root ganglion cells in mammals. We show here that motor neuron dendritic growth is highly oriented at early developmental stages. Initial dendrites grow in a bipolar orientation along the gray-white border zone and then give off branches into developing white matter. Between E14 and E15 dendrites grow into the gray matter in a pattern that differs between limb and axial motor neurons. Dendrites of axial motor neurons project dorsally along the midline whereas dendrites of limb motor neurons project directly medially. In contrast with the developmental sequence in frog (Jackson and Frank; J.Comp.Neurol. 255:538), we find that dendrites are growing into appropriate regions prior to the arrival of afferent fibers.

At E16 incoming Ia afferent axons segregate in the intermediate region of the cord. Afferents to axial motor neurons have their initial interaction with dorsally-directed dendrites along the midline and grow along these dendrites toward the somata. Afferents to limb motor neuorns contact medially directed dendrites and are guided to the lateral motor pools. Starting at E17 fibers branch repeatedly and elaborate boutons. Somata and proximal dendrites are densely invested whereas distal dendrites, dendrites that project into the white matter and dendrites that cross the midline are spared.

We conclude that, in mammals, motor neuron dendrites grow into regions of contact prior to the arrival of Ia afferent fibers. Afferents to limb and axial motor neuorns find appropriate synaptic partners by contacting different dendritic projections in the intermediate region of the cord. Finally, branching and elaboration of boutons does not occur until afferent fibers are in the region of the motor pools. These results suggest a major role for the target motor neuron in regulating the growth and branching of Ia afferent axons.

463.7

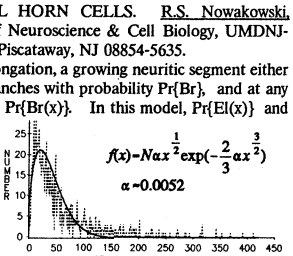
DENDRITIC DISTINCTIONS BETWEEN CALLOSAL AND SUBCORTICALLY PROJECTING PYRAMIDAL NEURONS DEVELOP FROM AN INITIAL COMMON MORPHOLOGY BY ELIMINATION OF EXUBERANT APICAL DENDRITES. S.E. Koester and D.D.M. O'Leary, Depts of Neurosurgery and Anatomy & Neurobiology, Washington Univ Sch Med, St. Louis, MO 63110

In adult rats, subcortically projecting pyramidal neurons, which are restricted to layer 5, have apical dendrites that arborize extensively in layer 1, whereas layer 5 callosal neurons do not send dendrites to layer 1 and are referred to as short pyramids (Hallman et al. 1988, J Comp Neurol 272:149; Games & Winer 1988, Hearing Res 34:1). To examine the development of the short pyramids, we placed DiI into one cortical hemisphere of a series of rat brains that had been aldehyde fixed at successive embryonic and postnatal ages (E22 = P0). Retrogradely labeled cells in contralateral cortex were examined in coronal sections several months later. In the embryonic cases, the retrogradely labeled cells are likely to be layer 5 neurons since most superficial callosal cells have not extended an axon into the contralateral cortex. Postnatally, layer 5 can be clearly discerned. At ages ranging from E19 to P4, virtually all deep callosal cells extend dendrites into layer 1. At the earlier ages, many of these dendrites are tipped with growth cones, while at the later ages most branch extensively in layer 1. Although there seem to be some subtle differences, at early postnatal stages layer 5 callosal neurons are similar in overall form to their subcortically projecting counterparts retrogradely labeled with DiI from the superior colliculus of age-matched pups. Thus, dendritic distinctions between these two classes are sculpted postnatally from an initial common dendritic morphology in a projection class-specific manner. These observations contrast with our findings that the projection distinctions between callosal and subcortically projecting layer 5 neurons are generated by class-specific axon growth rather than by a selective pruning of initially exuberant axons (Koester & O'Leary 1989, Soc Neurosci Abstr 15:960). Support: NEI grant EY07025, McKnight Foundation, NSF Predoctoral Fellowship.

463.8

A SIMPLE STOCHASTIC MODEL OF NEURITIC ELONGATION ACCURATELY DESCRIBES THE DISTRIBUTION OF DENDRITIC SEGMENT LENGTHS IN DORSAL HORN CELLS. R.S. Nowakowski, N.L. Hayes and M.D. Egger. Dept. of Neuroscience & Cell Biology, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ 08854-5635.

In our stochastic model of neuritic elongation, a growing neuritic segment either elongates with probability Pr{El} or branches with probability Pr{Br}, and at any given segment-length x, Pr{El(x)} = 1 - Pr{Br(x)}. In this model, Pr{El(x)} and Pr{Br(x)} are functions **only** of segment-length and not of branch order or of total distance from the soma. The histogram (broken line in inset) shows the number of segments/segment-length for N = 1236 measured dendritic segments (in bins of 1 μm) combined from 17 feline dorsal horn neurons labelled intracellularly with HRP. Each segment-length = the distance from the soma or from a branch point to the next branch point or to the termination of the process. The illustrated continuous curve is a predicted frequency distribution derived from one of several possible differential equations in which Pr{Br(x)} = g(x), when g(x) is a generally increasing function of x. This means that immediately after branching, when x is small, Pr{Br(x)} is small, but generally as x increases, Pr{Br(x)} increases. Many such derived functions, corroborated by our computer simulations of rules for dendritic growth, provide good fits to the collected data. Our model implies that much of the variation of dendritic segment-lengths may be attributable to complementary probabilities of elongating and branching which change as a function of **distance grown from the last branch point.** This has significant implications for understanding the development of branching patterns in dendritic trees.



463.9

DEVELOPMENT OF CORTICAL SPINY STELLATE CELLS: RETRACTION OF A TRANSIENT APICAL DENDRITE.
A. Peinado and L. C. Katz. Laboratory of Neurobiology, The Rockefeller University, New York, NY.

The local circuit interactions in which a cortical neuron participates are partly determined by the intralaminar and interlaminar extent of its dendritic tree. Mammalian cerebral cortex contains two broad classes of spiny excitatory neurons: pyramidal cells, which are found in all areas and all layers, and stellate cells, which predominate in layer 4 of primary sensory areas. Spiny stellates are distinguished from pyramids and star pyramids by the absence of an apical dendrite to more superficial layers. To assess the influence of thalamocortical afferents on the emergence of stellate and pyramidal cell morphology we examined the development of neurons in layer 4 of the rat's primary somatosensory cortex. Dendrites were visualized by intracellular injection of Lucifer Yellow in coronal brain slices from rats at postnatal days 2 to 20 (P2-P20). To identify layer 4 *in vitro*, thalamocortical afferents were labeled by anterograde transport of rhodamine injected into the ventrobasal thalamus.

Between P2 and P7 over 90% of the stained neurons in layer 4 (n=90) had an apical process that reached layer 1, where it usually branched at least once. Virtually no spiny stellate cells were apparent. After P7, the number of spiny stellate neurons increased such that by P10 fewer than 30% (n=69) of injected neurons extended an apical process beyond the layer 3/4 boundary. Activity in the thalamocortical afferents provides a critical cue for this remodeling: when we examined the morphology of neurons between P10 and P20 after cutting the infraorbital nerve unilaterally at birth, we found a far greater proportion of neurons (64%; n=81) extending an apical dendrite beyond the 3/4 laminar boundary. Taken together these results suggest that retraction of a transient apical dendritic process initially present in all spiny neurons accounts for a major morphological distinction between the two main classes of excitatory cortical neurons. This transition appears to be controlled at least in part by afferent input.
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463.10

CEREBELLAR MOSSY FIBER, BUT NOT CLIMBING FIBER, ELONGATION IS INTERRUPTED BY TARGET GRANULE NEURONS IN VITRO.
D.H. Baird, C.A. Baptista, M.E. Hatten, and C.A. Mason Dept. of Pathology, Coll. of Physicians and Surgeons of Columbia Univ., New York, N.Y. 10032.

We have devised an *in vitro* system to study the specificity of axon-target interactions. Explants of the major cerebellar afferent systems, pontine nuclei for mossy fibers, and inferior olivary nuclei for climbing fibers, were co-cultured with purified granule neurons from rat. Explants were dissected from mice at stages when their afferents invade cerebellum (P0 for pontine, and E16-17 for olivary explants) and neurites visualized with the mouse-specific monoclonal antibody M6 (Lund et al., *J. Comp. Neurol.* 247:439,1986). On laminin or poly-lysine substrates or on monolayers enriched for cerebellar astroglia, both pontine and inferior olivary explants displayed extensive neuritic outgrowth. In contrast, pontine explants grown on monolayers of their targets, granule neurons, extended many short neurites which terminated on cells close to the explant. This 'stop signal' is abolished by light fixation of the granule cell monolayer. Granule neurons do not act as a stop signal when plated far from, or on a coverslip above, pontine explants. Together with time-lapse video observations, these results indicate that the stop signal requires living target neurons and is contact-mediated. In addition, the stop signal provided by granule neurons is afferent-specific, at least within the cerebellum. Inferior olivary axons, which innervate Purkinje cells as climbing fibers, elaborate long neurites on granule cells. We are further exploring the specificity of the stop signal by co-culturing both types of afferents with Purkinje cells (Baptista et al., this volume). Supported by NS 16951 to C.M. and NS 08761 to D.B.

463.11

A SPECIFIC METALLOPROTEINASE INHIBITOR HAS TRANSIENT EFFECTS ON ACTIVITY OF NEURITES FROM EMBRYONIC CHICK NEURAL RETINA CELLS *IN VITRO*.
Joel B. Sheffield and Erik Dehlinger. Department of Biology, Temple University, Philadelphia, PA 19122

The developing neural retina expresses a set of extracellular proteases. When retina cells are cultured on a fluorescent gelatin substrate, growing fibers digest substrate in their paths. We suggest that the proteases are used by the tips of growing fibers to allow them to migrate within the mass of the tissue *in vivo*. We have examined the effects of an inhibitor, stereoisomer 1 of HS-Leu-Phe-Ala (HS-LFA) Darlack et al., J. Biol. Chem., 1990). Embryonic day 10 retinal cells were dissociated and plated at high density (10^6 ceils/cm^2 of surface area) on untreated plastic or glass substrates in medium containing 10% FBS. On the second day, when processes had begun to grow out, the medium was changed to one containing 10 μM HS-LFA. The behavior of the cells was monitored by time lapse video microscopy. Introduction of the inhibitor caused a cessation of both spike extension and growth cone ruffling, while control media had no effect. The behavior of growth cones was in sharp distinction to that of the cytoplasm, which continued in activity throughout. The inhibition of activity continued for between three and five hours, and the growth cones then gradually recovered their motility. By the end of the period, it appeared as if the culture had been unaffected by the treatment. Two other potential inhibitors were tested. Bathophenanthroline sulfonate, an inhibitor that chelates zinc from the active site of metalloproteinases, was toxic. Recombinant human TIMP (Synergen, Inc.) was inactive. The effects of HS-LFA are consistant with the concept that proteases play a significant role in fiber outgrowth from neural retina cells.

463.12

AXON TRAJECTORY-SPECIFIC DOMAINS IN THE HINDBRAIN OF THE CHICKEN EMBRYO.
J.C.Glover. Institute of Physiology, Univ. of Oslo, 0162 Oslo, Norway

The spatial relationships among brainstem neurons that project axons along different trajectories was determined by injecting retrograde tracers into developing axon tracts in 4-5d chicken embryos. Neurons projecting along a specific trajectory were in general organized into one or a few coherent clusters, with the clustering pattern being characteristic for each rhombomere. Injecting different fluorescent tracers into appropriate tracts allowed direct comparison of ipsi- and contra-projecting clusters of reticulospinal, vestibulospinal, and vestibulo-ocular neurons. This showed that such clusters occupy different, often segregated domains, indicating a spatiotopic relationship between soma position and axon trajectory. Whether soma position is involved in determining axon trajectory remains to be seen. Lineage analysis is being performed to determine the extent to which clonal relationships and migration patterns contribute to the formation of trajectory-specific domains.

463.13

THE POSITION OF THE MOTOR NERVE TRUNKS TO THE EMBRYONIC ABDOMINAL MUSCLE IN *XENOPUS* IS DETERMINED BY SOMITE-DERIVED CELLS.
Kathryn Lynch, Uniformed Services University, Bethesda, MD 20814-4799.

It is not yet known what determines the course of major vertebrate nerve trunks. I have used microscopy (EM and LM) to examine the influence of myogenic cells on the distribution of the first motor nerve trunks to the embryonic abdominal muscle (EAM) in *Xenopus*. The first motor axons to the EAM arise from spinal nerves 2-9, and follow clusters of somite-derived myogenic cells that migrate ventrally between epidermis and lateral plate. At N&F St 39-40, the cells in each cluster fuse to form a segment of the EAM. At St 40, trunks of axons are apposed to the boundaries between muscle segments. Because the myotube tips stain for AChE, the narrow boundary zones are visible in AChE-reacted embryos, normally as six dark transverse lines across the broad flat muscle. However, if a premigratory cell cluster is partially or wholly ablated, the regular segmentation of the muscle is disrupted; an intersegmental boundary may be missing or intermittent, with short sections at different rostrocaudal levels. Nerve trunks in such cases are still closely associated with the boundary zones. When a boundary is missing, two trunks may converge on a single boundary. Nerve trunks zigzag between sections of the intermittent boundary zones. Thus the position of the first nerve trunks is determined by the location of the segmental boundaries. This in turn seems to be a function of the somite-derived myogenic cells, not the en vironment into which they migrate. Nerve trunk position in older embryos and after ablation of more than one premigratory cell cluster will also be examined.

464.1

DEVELOPMENT OF EXCITABILITY IN DISSOCIATED CAT RETINAL GANGLION CELLS. I.Skaliora*, L.M.Chalupa and R.P.Scobey. Depts of Psychology, Neurology and Neurobiology Graduate Group, Univ. of California, Davis CA 95616.

We are studying the development of excitable membrane properties in acutely isolated retinal ganglion cells from fetal and postnatal animals. Cells are enzymatically dissociated in a papain solution and kept in suspension at 10°C for up to 48 hours after eye removal. Ganglion cells are identified by retrograde labeling with rhodamine beads previously injected into the LGN and SC. The whole cell and the perforated patch variations of the patch clamp technique are used to obtain voltage and current clamp recordings respectively.

Cells from postnatal animals (1 week to 3 months of age), invariably manifest spikes in response to depolarizing steps of current, and generate sustained- and transient-type firing patterns. At embryonic day 40 (E40), the earliest age recorded so far, cells do not manifest impulse activity at any stimulus intensity and the net inward currents are barely detectable. By E45 there is a large increase in inward current amplitudes. At this age, sodium mediated spikes are apparent in most cells, as indicated by their blockade following TTX application. Whereas increasing proportions of the cell population display spike activity as a function of age, there are no significant differences in the duration, rate of rise or amplitude of individual spikes. The data suggest that, in cat retinal ganglion cells, the basic features of excitability are established early in development, shortly before the onset of segregation of their projections into eye specific layers.

(supported by EYO 3991 from the NEI)

464.2

LOCALLY CORRELATED SPONTANEOUS ACTIVITY OF NEURONS IN THE DEVELOPING MAMMALIAN RETINA. R.O.L. Wong, M. Meister*, D.A. Baylor and C.J. Shatz. Dept. Neurobiology, Stanford Univ. Sch. Med., Stanford, CA 94305.

Spontaneous electrical activity in retinal ganglion cells during early development is thought to play a key role in guiding the formation of orderly connections in the visual system. This activity is essential for the progressive segregation of retinal axonal terminals into eye-specific layers within the lateral geniculate nucleus. Using a multielectrode recording technique, we previously established that cells in isolated immature cat and ferret retinae fired action potentials in bursts that occurred almost synchronously, and that this activity frequently spreads across the piece of retina as a wave travelling at about 100 µm/sec (Meister et al., Invest. Ophth. Vis. Sci. 31 (Suppl.), 115, 1990). In contrast, there were no distinct bursts or wave-like activity in the adult retina. Rather, adult cells showed continuous spontaneous firing in darkness and gave brisk ON and OFF responses to light, as found *in vivo*. In further studies, periodic bursts were also recorded intracellularly from cells in immature retina which were subsequently identified as retinal ganglion cells by dye injection. Thus, it is likely that some of the cells recorded with the multielectrode array are ganglion cells, suggesting that spontaneously generated patterned activity of these cells may play an instructive role in the segregation of their axons in target structures. The mechanism underlying the synchronous bursting activity during development and the factors responsible for its disappearance by adulthood are currently being investigated.

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464.3

NMDA ANTAGONISTS DISRUPT NORMAL ON/OFF SUBLAMINAR SEGREGATION OF FERRET RETINOGENICULATE AXONS. J. Hahm, R. B. Langdon and M. Sur. Department of Brain & Cognitive Sciences, M.I.T., Cambridge, MA 02139.

To examine the role of NMDA receptors in the segregation of retinal afferents into ON and OFF sublaminae in the ferret LGN, we chronically applied antagonists during the period of normal segregation. Previously, we have shown that retinal axon arbors become restricted to ON or OFF sublaminae between two and three weeks postnatal (Hahm & Sur, Soc. Neurosci. Abst. 14:460, 1988). At postnatal day 14, ferret kits were chronically infused for one week with one of the NMDA antagonists, APV (0.5mM - 1.6mM), MK-801 (1.23mM - 4.74mM), or CPP (0.063mM - 79.3mM). Control animals received infusion of saline (0.9%). Compounds were administered via an osmotic minipump (1µl/hr), by subcutaneous implant alone (MK-801, CPP) or through a cannula inserted into the brain rostral and medial to the LGN and attached to the minipump (APV, saline).

Intraocular injections of HRP/WGA-HRP (20%/2%) at three weeks of age revealed that APV and MK-801 prevented retinal afferents from segregating into ON/OFF sublaminae. CPP disrupted segregation to block complete separation of sublaminae.

Labeling of single axons *in vitro* in CPP treated animals showed that in addition to incomplete sublaminar restriction of retinal axon arbors, short side branches had sprouted along the main trunk of the axon. Normally, side branches are seen in the first postnatal week, but have disappeared by two weeks of age.

These results suggest that NMDA receptors mediate activity-dependent segregation in the ferret retinogeniculate projection. Since NMDA receptors are crucially involved in retinogeniculate transmission, the effects of NMDA blockade may be a consequence of blocking transmission in the LGN during development.

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464.4

PRENATAL DEVELOPMENT OF LGN NEURON DENDRITES DURING SEGREGATION OF EYE INPUTS. A. Ghosh, M. B. Dalva* and C. J. Shatz, Dept. of Neurobiology, Stanford Univ., Stanford, CA 94305

To examine postsynaptic changes occurring in the lateral geniculate nucleus (LGN) during segregation of retinal afferents, we have studied the morphological changes in LGN projection neuron dendrites between E43 and E57 in the cat. LGN neurons were retrogradely labeled by injecting diI into the primary visual cortex in aldehyde-fixed brains. At E43, when retinal afferents have not yet formed terminal arbors, the LGN projection neurons already have relatively extensive dendrites. Most cells have several primary dendrites arranged in a fusiform fashion (total dendritic length=654±360µm). During the following two weeks both total dendritic length and higher order dendritic branching continue to increase (at E57 total dendritic length=1049±304µm). To determine whether action potential activity influences dendritic development during ocular segregation, we studied LGN cell morphology following 2 week minipump infusion of tetrodotoxin (TTX) between E43 and E57. We verified that the TTX infusion was successful by injecting diA into the optic tract of the same animal: as expected, retinal terminals had not segregated into eye specific layers. Despite this effect on axon arbors, a study of 23 cells from a TTX treated LGN shows that LGN dendritic development is essentially normal in morphology (total dendritic length=965±298µm). These observations show that there is significant dendritic development during ocular segregation in the LGN; however the pattern of dendritic growth during this period seems to be independent of activity. Thus it appears that within the same structure, blockade of action potential activity can markedly alter the morphology of presynaptic axons while having little effect on postsynaptic dendrites. (NSF BNS 89-19508 to CJS and T32 HD07249 to AG.)

464.5

FORMATION OF AFFERENT PROJECTIONS IN ORGANOTYPIC SLICE CULTURES FROM RAT VISUAL CORTEX. M. Götz(1), N.Novak*(1), V.Staiger*(2), T.Bonhoeffer(2) and J. Bolz(1);(1) Friedrich-Miescher Labor der Max-Planck Gesellschaft and (2) Max-Planck Institut für biologische Kybernetik, 7400 Tübingen, FRG.

Thalamic afferents form very specific connections with their cortical target cells. In order to learn about mechanisms involved in the development of these connections we used a thalamocortical in vitro system. We prepared slice cultures from the visual cortex of young rats (P0 - P8) and cocultured them with slices from the lateral thalamus (E16 - P5). Thalamocortical connections were traced with DiI in cocultures after 4-14 days in vitro. As reported previously, cortical slices survive for several weeks in vitro and the different cell types continue their morphological and neurochemical maturation (Caeser et al., Exp. Brain Res.77,234,1989; Götz and Bolz, Neurosci. Lett. 107,6,1989).To examine the laminar organization of the cortical cultures, cells of different layers were labeled at their birthdates by injecting timed pregnant rats with BrdU. After 1-3 weeks in culture the laminar distribution of labeled cells matched the in vivo situation at the corresponding age. This was also true for the earliest generated neurons situated in the so called "subplate". It has been proposed that subplate cells serve as transient targets for thalamic afferents before they disappear. We found that many subplate cells survive both in vivo and in slice cultures where no afferents are present. In cocultures fibers from thalamic slices obtained from animals up to P2 grew into the cortical explant and terminated in layers 4 and 3, but not in the subplate zone. This specific ingrowth was established whether the thalamic slice was placed at the white matter or at the pial side of the cortex slice. Electrophysiological and optical recording experiments showed that the thalamocortical connections are functional. Thus thalamic afferents form specific connections with their cortical targets in vitro without a "waiting period" in the subplate zone.

464.6

FORMATION OF EFFERENT PROJECTIONS IN ORGANOTYPIC SLICE CULTURES FROM RAT VISUAL CORTEX. J. Bolz, N. Novak* and M.Götz. Friedrich-Miescher Labor d. Max-Planck Gesellschaft, 7400 Tübingen, FRG.

Cortical projection neurons are segregated in cortical layers and there is a close relationship between cell morphology and efferent target. We studied cortical projections in vitro using the culture system described in the preceeding abstract. Slice cultures of the visual cortex were prepared from 0 to 2 day old rats, an age at which the axons of cortical projection neurons have not yet reached their targets. The superficial layers of the cortex are not formed at that time, but BrdU-prelabeling showed that also cells of layer 2/3 are located at their appropriate position after 1-3 weeks in vitro, suggesting that migration continues in the slice cultures. Cortex slices were cocultured either with a slice from a subcortical target (lateral thalamus or superior colliculus) or with another slice of visual cortex. Retrograde tracing with DiI after 4-14 days in vitro showed that almost all cells labeled from subcortical explants were located in the deep cortical layers, exactly where these cells are found in vivo. Cells in the superficial layers did not innervate these targets, but did project into cocultured cortical slices. In cortex-cortex cocultures, some projecting cells were also located in the deeper layers, but their morphology was different from subcortical projecting neurons. Similar differences have been observed in vivo (Hübener and Bolz, Neurosci.Lett.94,76,1988). Thus the laminar origin and cellular morphology of cortical projections formed in vitro are target dependent. Moreover, these specific connections were established irrespective of whether the explanted targets were placed towards the white matter or the pial side of the cortical cultures. With ectopically positioned targets, the axonal trajectories of the projecting cells were always oriented towards their targets and not restricted to particular pathways. This suggests that chemotropic attraction may play an important role in the formation of connections between cortical neurons and their efferent targets.

464.7

POSTNATAL DEVELOPMENT OF GENICULOCORTICAL SYNAPSES IN AREA 17. M.J. Friedlander, J.C. Anderson*, C. DeHay*, K.A.C. Martin* and J.C. Nelson*. Neurobiology Research Center, University of Alabama at Birmingham, Birmingham, Al, 35294, and Anatomical Neuropharmacology Unit, Department of Pharmacology, Oxford University, U.K.

Individual geniculocoritcal Y-axons in the cat increase the extent of their innervation of layer 4 in cortical area 18 between 5 postnatal weeks of age and adulthood. During this same period, the individual boutons enlarge, increase their spacing and acquire more postsynaptic targets (Friedlander and Martin, J. Physiol. 416:183-213). To date, similar developmental data are not available for cortical area 17. A comparison of the developmental processes between these two cortical areas is of interest since the type of competitive interactions that occur may differ. In layer 4 of area 18 (innervated only by Y-axons), competition between developing geniculocortical axons is limited to binocular interaction. However, in layer 4 of area 17, competitive interactions may occur between functional cell types (X- vs. Y-cells), as well. In the present study, we used intracellular injections of horseradish peroxidase to label individual physiologically indentified geniculocortical X- and Y- axons that project to area 17 in kittens at 5 postnatal weeks of age and adult cats. The extent of the arborizations in layer 4 (n=5) were digitized and over 100 boutons (≈25 each for 4 or the 5 axons) were serially sectioned and fully reconstructed for electron microscopical analysis. The kitten axons terminated primarily in layer 4 with occasional branches innervating layer I (X-axon). The individual kitten boutons (both X- and Y-) were smaller than their adult counterparts, they formed asymmetric contacts primarily on dendritic spines and they generally contacted only a single, postsynaptic target. Occasionally (5%) kitten boutons made no synaptic contacts. Thus, our preliminary observations on the development of the ultrastructure of geniculocortical synapses for X- and Y-axons in area 17 suggest similar processes to those observed for Y-axons in area 18.

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464.8

DENDRITIC CHANGES IN GRANULE CELLS OF OWL VISUAL CORTEX FOLLOWING VISUAL DEPRIVATION. J.D. Pettigrew and I.C. Gynther. Vision, Touch and Hearing Research Centre, The Univ. of Queensland, Qld. 4072, Australia.

Investigation of the cortical events underlying monocular deprivation is facilitated by two, technically favorable aspects of the owl's visual cortex. Firstly, the owl's LGN is monocular so that anterograde monosynaptic labelling of the whole input array from one eye is possible. Secondly, ocular dominance columns are not normally present in the owl but appear as a result of deprivation. The fine morphology of granule neurons with known relationships to ocular dominance column boundaries was studied in visual cortex of deprived owls after Lucifer Yellow (LY) microinjection in fixed brain slices. Injections were guided towards ocular dominance columns and their boundaries by rhodamine fluorescence from anterogradely-transported TRITC. Antibodies against LY were used to convert injected cells to a permanent, non-fluorescent form. Spiny stellate cells lying on or close to the boundaries were found to have dendrites oriented with respect to the column boundary. In contrast, comparable neurons from elsewhere in the granular layers had dendrites which were radially symmetrical. A similar difference may apply to the non-spiny stellate cells, but our sample of these neurons is not yet sufficiently large. In addition to the changes induced at column borders as a result of deprivation, we also found alterations of the dendritic trees of stellate cells at another, naturally-occurring boundary, that between the monocular and binocular segments of the granular layer, where there would be natural competition between inputs from the ipsilateral and contralateral eye during development.

464.9

DEVELOPMENT OF INTERLAMINAR CONNECTIONS OF LAYER 4 NEURONS IN CAT STRIATE CORTEX. L.C. Katz and E.M. Callaway. Laboratory of Neurobiology, The Rockefeller University, New York, NY 10021.

Clustered horizontal collaterals of layer 2/3 neurons link iso-orientation columns in cat striate cortex. During development, crude clusters emerge at postnatal day 8 (P8) and are refined over the next 3-4 weeks. Because development of horizontal connections is activity dependent (see following abstract) and spiny cells in layer 4 form a major excitatory input to layer 2/3, this input might direct the formation or refinement of crude clusters. Consequently, we examined the development of axons of layer 4 spiny neurons after intracellular staining in slices of cat striate cortex. At P5-8, no cells (0/14) extended axon collaterals into layer 3, but very short (<100μm) collaterals sometimes emerged from a main descending axon within layers 5, 6 and/or the subplate. The first collaterals crossed into layer 3 at P11; about 20% of cells (4/19) had a single collateral extending 50-200μm beyond the laminar boundary. By P15, 80% of cells (16/20) projected to layer 2/3. This proportion was similar to that observed in the oldest animals, but the axonal arbors did not achieve an adult-like extent or appearance until P26. In contrast to the extensive increase in projections to layer 2/3, collaterals within layers 5 and 6 remained short and sparse, and reached roughly adult levels by P15. Since layer 4 spiny neurons do not project into layer 2/3 prior to the appearance of crude clusters, activity relayed through this pathway may not play an important role in cluster emergence. Layer 2/3 neurons might, however, receive orientation information directly from geniculate afferents or from functional connections between layer 4 neurons and the basal dendrites of deep layer 3 neurons. The emergence of projections from layer 4 to 2/3 is closely correlated to the timing of both the refinement of clustered connections and increased visual responsiveness of layer 2/3 neurons, suggesting that activation by layer 4 inputs could play an important role in cluster refinement.

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464.10

BINOCULAR DEPRIVATION REDUCES THE SPECIFICITY OF CLUSTERED HORIZONTAL CONNECTIONS IN CAT STRIATE CORTEX. E. M. Callaway and L. C. Katz. Laboratory of Neurobiology, The Rockefeller University, 1230 York Avenue, New York, NY 10021.

In the striate cortex of adult cats, pyramidal neurons in layer 2/3 have long, intrinsic horizontal axon collaterals that specifically interconnect iso-orientation columns (Gilbert & Wiesel, J. Neurosci. **9**: 2432). Thus, retrograde tracer injections result in clusters of labeled cells spaced about 1mm apart and extending several mm from the injection site. We previously demonstrated that during the first postnatal week retrograde labeling is unclustered and that a crudely clustered distribution appears during the second week. These crude clusters are subsequently refined by the selective elimination of axon collaterals projecting to incorrect orientation columns (J. Neurosci. **10**: 1134). Here we report that binocular lid suture, which prevents patterned visual activity, did not prevent the initial *emergence* of a crudely clustered pattern of retrograde label. Binocular deprivation did prevent the *refinement* of clusters, indicating that horizontal axon collaterals projecting to incorrect orientation columns were maintained well past the age when they are normally eliminated. Analysis of intracellularly stained layer 2/3 pyramidal cells from binocularly deprived cats revealed that their axonal arbors underwent many normal developmental changes. In particular, rearrangement of the radial positions of long horizontal collaterals and addition of finer branches to distal portions of those collaterals were both observed. Consistent with the lack of cluster refinement, however, the radial distribution of horizontal collaterals from binocularly deprived cells was significantly less clustered than normal. Thus, binocular deprivation did not arrest development at an immature stage, but reduced the specificity with which axon collaterals were added or eliminated. We conclude that patterned visual activity provides a critical cue for the establishment of specific horizontal connections.

Supported by EY06128, EY07960, and the L. P. Markey Charitable Trust.

464.11

POSTNATAL DEVELOPMENT OF INTRACORTICAL CONNECTIONS IN HUMAN VISUAL CORTEX. A.Burkhalter, K.L.Bernardo and V.C.Charles. Dept. of Neurosurgery and McDonnell Center for Higher Brain Function, Washington Univ. Sch. of Med., St. Louis, MO 63110.

Different visual functions emerge at different ages and with different time courses. We have investigated whether such behavioral changes are correlated with the development of specific intracortical circuits. Tracing with the fluorescent dye, dil, was used in postmortem fixed brains of human infants (newborn to 5 years of age) to visualize connections within and between the visual cortical areas V1 and V2.

At birth the connections between V1 and V2 are immature, in that unbranched horizontal fibers are seen only in deep layers and do not show the lamination patterns of forward and feedback projections. During the first 2 postnatal months fibers occur in middle and superficial layers and clustered vertical collaterals emerge. The development of forward and feedback projections proceeds in parallel and the reciprocal circuit between V1 and V2 reaches maturity at 1-2 years of age. At birth the local connections within columns of V1 and V2 appear mature, unlike the local long-range projections within upper layers (Burkhalter and Bernardo, Soc.Neurosci.Abstr. 15:2, 1989). Within V2 horizontal fibers in layer 2/3 emerge during the first postnatal month and develop before those in V1. At 4 months they show terminal clustering similar to adults. In contrast, very few horizontal fibers are seen in upper layers of V1 until 2 years of age.

These results suggest that prominent intracortical circuits are formed after basic spatial, temporal and binocular visual functions have reached maturity (Boothe et al., Annu.Rev.Neurosci. 8:495, 1985).

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464.12

C-FOS IMMUNOREACTIVITY IN CAT VISUAL CORTEX. F. Vandesande (1), L. Arckens* (1,2), U. Eysel (3) and G.A. Orban (2). (1) Lab. Neuroendocrinologie, K.U.Leuven, Naamsestraat 59, B-3000 Leuven, Belgium. (2) Lab. Neuro-en Psychofysiologie, K.U.Leuven, Campus Gasthuisberg, B-3000 Leuven, Belgium. (3) Dept. Neurophysiology, Ruhr Universität Bochum, Universit 150, D-4630 Bochum 1, F.R.G.

Cats were perfused under Nembutal anaesthesia with 2 % paraformaldehyde and 1.25 % glutaraldehyde fixative. Brains were sectioned and the sections stained with a polyclonal antibody specific for the c-fos P10 region (Medac) using the PAP technique. C-fos immunoreactivity (ir) was observed in the nuclei of numerous visual cortical cells (area 17 and 18). The ir was lamina specific in that in the middle layer (IV) fewer nuclei were stained.

Double staining with anti-GABA and anti-c-fos revealed very few labeled cells suggesting that most c-fos ir neurons are non-GABAergic.

The effect of visual experience on c-fos ir in visual cortex was assessed by making bilateral retinal lesions and comparing parts of cortex subserving central and peripheral vision and by keeping cats under anaesthesia. Preliminary results indicate that visual stimulation has a short living effect (a few hours) which is lamina and area specific, while the effect of visual deprivation is noticeable only after several days and consists in a decrease of the overall level of staining rather than a change in the laminar pattern.

464.13

PARVALBUMIN IMMUNOREACTIVITY IN THE CAT VISUAL CORTEX DURING NORMAL DEVELOPMENT AND AFTER VISUAL DEPRIVATION. J. R. Naegele[1], K. Fox[2] and N. Daw[2]. Depts. of Ophthalmology and Visual Sciences, Yale University School of Medicine, New Haven CT. 06510[1] and Physiology and Cell Biology, Washington University School of Medicine, St. Louis, MO. 63110[2].

Parvalbumin (PV) is a calcium-binding protein expressed in about 70% of GABA neurons in the mammalian cerebral cortex. Although its exact function is not yet known, PV may effect firing patterns or levels of excitation in fast-spiking types of GABA neurons. One of the most numerous types of PV-immunoreactive (PV-ir) cortical neurons are the basket cells. We have used immunocytochemical staining to study the expression of PV in cortical area 17 in normal and dark-reared kittens. PV was localized with a monoclonal antibody generated against carp muscle parvalbumin (mAb 235, Celio et al., '89, Cell Calcium 9: 81-86) and as controls, we used a panel of molecular markers for basket cells including mAb VC1.1 and the lectin VVA. On the day of birth (P1), PV-ir cells were restricted to layer 6 and the subplate zone. By P14, PV was expressed in layers 4-6 and in the subplate. By P21, PV-ir cells were present in all cortical layers except layer 1. During subsequent maturation, numbers of PV-ir cells increased within layers 3 and 4 and decreased in layers 5 and 6. In dark-reared kittens, the number and intensity of PV-ir cells was markedly reduced, however littermates exhibited normal patterns of PV-ir when exposed to light for 4-10 days, after dark-rearing. Dark-reared kittens also showed deficits in receptive field properties but light exposure resulted in recovery of normal receptive field properties. These findings raise the possibility that in dark-reared animals, deficits in receptive field properties in area 17 may be related to lower levels of PV in the basket cells. (Supported by the Klingenstein Foundation).

CIRCUITRY AND PATTERN GENERATION III

465.1

MEDULLARY SLICES THAT GENERATE RESPIRATORY OSCILLATIONS *IN VITRO*. Jeffrey C. Smith, John J. Greer & Jack L. Feldman, Systems Neurobiology Lab., Department of Kinesiology, UCLA, LA, CA 90024 -1586.

We identified a limited region of the ventrolateral medulla critical for generation of respiratory rhythm (Smith et al., Soc. Neurosci. Abs. 15:505, '89). This region, called the preBötzinger region, was identified by combining extracellular recording and precision microsectioning of neonatal rat brainstem-spinal cord preparations *in vitro*. To further test the hypothesis that the region contains cell populations for rhythmogenesis, we cut medullary slices to determine if this region in isolation can spontaneously generate a respiratory oscillation. Brainstem-spinal cord preparations were mounted in a vibratome bath (*ibid.*), sectioned to within 150 μm of the rostral boundary of the critical region, and a single 400-500 μm thick transverse medullary slice containing the critical region was cut. The slices preserved rootlets of glossopharyngeal and hypoglossal nerves, allowing recording of oscillatory motoneuron activity in addition to medullary neuron activity within the slice. These slices contained the critical region and adjacent reticular formation regions, and excluded all other medullary regions hypothesized to contain cells involved in rhythmogenesis (e.g. Ominaru et al., Brain Res. 445: 314, '88). The slices spontaneously generated a rhythmic motoneuronal output, demonstrating that they contained networks sufficient for both rhythm and (pre)motoneuronal discharge pattern generation. By retaining or excluding different amounts of the flanking regions at the slice boundaries, we obtained evidence for the following features of the spatial organization: (1) There is a cell population(s) at the rostral boundary of the critical region that provides an excitatory drive to the rhythm generating cells essential for the production of oscillatory activity; slices without these cells would not generate a spontaneous oscillation unless cells were depolarized by exogenous neuroexcitants, particularly excitatory amino acids acting at nonNMDA receptors. (2) The area bordering the caudal boundary of the critical region is necessary for motoneuronal burst pattern formation. This data is consistent with our model for pattern generation in which there are two functionally and spatially segregated components: (i) a cellular oscillator consisting of conditionally bursting pacemaker neurons requiring excitatory synaptic drive for rhythmogenesis; (ii) separate more caudally distributed populations involved in burst pattern formation that transform the rhythmic drive from the oscillator into a detailed spatiotemporal pattern of (pre)motoneuronal activity. Supported by NIH grants HL 40959 and HL 02204.

465.2

TIME SERIES ANALYSIS OF SPIKE PATTERNS IN CULTURED AND SIMULATED SMALL NEURONAL NETWORKS. J.M.Kowalski*, G. Albert*, and G.W. Gross**. Dept. of Physics, Dept. of Biological Sciences**, Center for Network Neuroscience, Univ. of North Texas, Denton, TX 76203.

Small neuronal networks (100-500 neurons) grown from dissociated mouse spinal cord as monolayer cultures on a photoetched multimicroelectrode matrix can be maintained for several months and monitored continually for several days. These networks exhibit spontaneous, highly patterned activity with extensive course-grained synchronization. Assuming that the underlying system's dynamics are deterministic, one may apply the Taken's embedding theorem to reconstruct the dynamic attractors and estimate their correlation dimension. We discuss the relevance of this method in the network setting, where the recorded variables are arbitrary smooth functions of the network state. It is stressed that the presence of a scaling law alone with small exponent for the correlation dimension cannot be considered as a "proof" that the underlying time series has a deterministic origin. Some other important "caveat's" in the method's application are discussed (selection of the representative scalar variable, error bounds, etc.). We model our networks as ensembles of coupled (via slow variables) Chay's neuromimes, where each unit may exhibit a full activity range (i.e. resting, periodic, quasiperiodic, and chaotic states). Observed network "self-ignition" and synchronization phenomena can be explained within this model. Calculated correlation dimension of such models is compared with those from experimental data.

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465.3

SEROTONIN ENHANCES SYNAPTIC FATIGUE IN NEURONAL CIRCUITS OF THE LEECH. P.S. Mangan and W. Otto Friesen. Dept of Biology, Univ. of Virginia, Charlottesville, VA 22901

Micromolar concentrations of serotonin (5-HT) elicit swimming activity in isolated leech ventral nerve cords in both normal animals (Willard, J. Neurosci. 1:936-944, 1981) and animals in which endogenous serotonin has been depleted by treatment with reserpine (Friesen et al., Soc. Neurosci. Abst. 14:384, 1988). Previously, 5-HT was reported to modify synaptic transmission between DI motor neuron 102 and central swim oscillator cell 115 (ibid.). We report here serotonin-induced modification of synaptic physiology in three motor neuron pairs of the leech swim circuit. The synapses examined were those between DI cell 1 and DE cell 3, VI cell 2 and VE cell 4, and cells 1 and 2.

Inhibitory synaptic transmission was monitored, prior to and following addition of 50 μM serotonin, in both untreated and reserpine-treated animals. The pre-synaptic cell was injected with depolarizing current pulses (1-3 nA, 1-2 s) and inhibitory post-synaptic potentials resulting from 10-20 identical pulses were recorded, digitized, and averaged. Synaptic fatigue increased substantially with 5-HT application in both untreated (30-fold) and reserpine-treated (80-fold) preparations as compared with controls (untreated, reserpine-treated; normal saline; pooled data from all synapses). Serotonin-induced fatigue was 30% greater in untreated preparations than in reserpine-treated preparations. Half-maximum fatigue was attained in 0.33s and 0.49s in untreated and reserpine-treated preparations, respectively. The increase in synaptic fatigue induced by elevated 5-HT could facilitate oscillatory activity in the reciprocally inhibitory interactions prevalent in the leech central swim network and thus provided the basis for serotonin-enhanced expression of swimming activity in the intact animal. Supported by grants NS08781 (P.S.M.) and NS21778 (W.O.F.)

465.4

GLUTAMATE: PUTATIVE NEUROTRANSMITTER IN THE FEEDING CENTRAL PATTERN GENERATOR OF HELISOMA TRIVOLVIS. E.M. McLean and A.D. Murphy. Dept. of Biological Sciences, University of Illinois at Chicago, Chicago, Illinois, 60607.

The feeding central pattern generator of Helisoma trivolvis consists of three subunits (S1-S3) that are independent interneuronal oscillators. These subunits provide excitatory drive to sets of motor neurons. Most feeding-related neurons receive post-synaptic potentials from the S2 subunit. Neurons that receive S2 IPSPs are hyperpolarized by bath application of glutamate or quisqualate, and neurons that receive S2 EPSPs tend to be depolarized by glutamate or kainate. Glutamate and kainate also stimulate robust patterned motor activity. The compound alpha-amino pimelic acid has both glutamate agonist and antagonist effects in this system. It elicits high frequency patterned motor activity, while gradually decreasing the amplitude of S2-driven psps. This work supports the hypothesis that the S2 interneurons are glutamatergic, activating kainate-like receptors at excitatory synapses and quisqualate-like receptors at inhibitory synapses.
(Supported by NIH Grant NS26145)

465.5

CRUSTACEAN CARDIOACTIVE PEPTIDE (CCAP): A HORMONE NEUROMODULATOR OF THE STOMATOGASTRIC NERVOUS SYSTEM.
Lawrence I. Mortin, Sybil T. Lockhart*, and Eve Marder.
Biology Department, Brandeis University, Waltham, MA 02254.

The nonapeptide crustacean cardioactive peptide (CCAP) was isolated and sequenced from the pericardial organs (PO) of the crab *Carcinus maenas* (Stangier et al., PNAS 84:575, 1987) We used a polyclonal antibody raised against synthetic CCAP (Dircksen & Keller, Cell Tissue Res. 254:347, 1988) to study CCAP-like immunoreactivity in the crab *Cancer borealis* and the lobster *Panulirus interruptus*. In *C. borealis*, this antibody stained many nerve fibers and terminals in the PO, but no staining was seen in the stomatogastric nervous system. In contrast, in *P. interruptus*, a pair of cells, one in each commissural ganglion (CG), showed CCAP-like immunoreactivity. Punctate varicosities, resembling neuropil-like structures, were stained in the lateral portion of each CG, as was a single through-fiber within the commissural connective. A single large cell shows CCAP-like staining in the esophageal ganglion.

Preliminary physiological experiments show that bath application of CCAP to the *in vitro* stomatogastric nervous system produces an ensemble of effects on the motor rhythms controlled by this portion of the nervous system, which controls the muscles of the foregut. Motor pattern changes were induced in both *P. interruptus* and *C. borealis* with 1 μM CCAP or less. These results suggest that a CCAP-like peptide may act as a neuromodulator of the central pattern generators of the crustacean stomatogastric nervous system.

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465.6

TWO TYPES OF IDENTIFIED MODULATORY NEURON SUBSERVE TWO TYPES OF FUNCTION: MAINTENANCE OR ALTERATION OF A RHYTHMIC MOTOR PATTERN IN CRUSTACEA.
F. Nagy, T. Bal*, P. Cardi* and M. Moulins. Lab. Neurobiologie et Physiologie Comparées, CNRS, Univ. Bordeaux I, 33120 Arcachon, France.

In isolated preparations of the crustacean stomatogastric nervous system, rhythmic activity of the pyloric CPG, and oscillatory properties of its neurons, are strictly dependent upon tonic modulatory inputs from higher centers. A central modulatory neuron (APM) was identified whose discharge can induce rhythmicity in a previously silent pyloric network. However, APM and several other modulatory neurons identified since, are generally not spontaneously active in vitro, and cannot provide the tonic input to maintain normal pyloric activity. We show in the lobster, *Homarus gammarus*, that this sustained modulatory function is fulfilled by a pair of commissural neurons, P and CP. In vitro, they are always (n=63) active with the pyloric network. Silencing them provokes immediate cessation of pyloric activity (n=6). When deprived of P and CP inputs, all pyloric neurons switch from cellular oscillators to passive neurons. When compared to APM, P and CP appear to exert significantly different effects. While all three modulators can induce oscillatory properties in previously passive pyloric neurons, only oscillations induced by P and CP resemble normal activity. Moreover, APM's effects long outlast the duration of its discharge, whereas those of P and CP do not. Finally, during spontaneous pyloric activity, depolarizing either P or CP to fire up to 35 Hz, activates but does not disorganize pyloric activity, whereas a brief discharge of APM at only 10 Hz lastingly transforms the pyloric pattern.

These results, therefore, indicate two classes of modulatory neuron: those like P or CP, whose sustained discharge maintains basic rhythmic activity of a CPG; and those like APM, with strong and longlasting effects but episodic discharge, which induce temporary alteration in the ongoing activity of a CPG.

465.7

IONIC CURRENT FINGERPRINTS IN LOBSTER STOMATOGASTRIC NEURONS.
D.K.Hartline, D.V.Gassie*, B.A.Tomiyasu* and B.R.Jones.
Békésy Laboratory of Neurobiology, Univ. of Hawaii, Honolulu, HI 96822

Two-microelectrode voltage clamp recordings were made in lobster (~0.5 kg *Panulirus* spp., 22° C) stomatogastric neurons to determine if unique ionic current patterns are associated with different identified cell types. Standard test pulses to 0 mV (with and without conditioning prepulses) revealed a transient outward current (A), and a calcium-dependent outward current (J) composed of fast (Jf), slow (Js) and maintained (Jm) kinetic components.

Current magnitudes in intact and ligated somata are statistically indistinguishable within a cell type. The pyloric group differs from most of the gastric group by having faster A inactivation and slower J inactivation. Differences within each group are shown by differences in A, J and total outward current. Figures show cell specific current patterns (magnitude of A *vs* magnitude of Jm) for pyloric (left figure) and gastric (right figure) neurons. Possibly significant deviations from cell-specific profiles occur.

Modeling studies suggest that large conductances and outward current sign reduce the space-clamp error (<10-15% at 0 mV) in intact somata.

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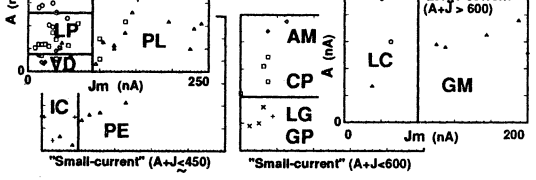

465.8

USE OF MODELING TO ANALYZE ORIGIN OF EMERGENT OSCILLATIONS OF THE GASTRIC MILL CPG IN THE LOBSTER STG.
P.F.Rowat and A.J.Mandell. Biology B-022, U.C. San Diego, La Jolla, CA 92093.

The gastric mill CPG oscillates at low frequency without a pacemaker cell. All component cells and physiological synapses are known, but strengths of ionic and synaptic currents are not well characterized due to limitations of voltage clamp techniques. Most gastric cells display plateau potentials and have conditional bursting properties. On the basis of its behavioral characteristics, we hypothesize that the network has the dynamics of a relaxation oscillator. Modeling and phase portrait analysis are used to analyze how synaptic and electrotonic connections and membrane currents contribute to emergence of network oscillations. Modeling software was developed in which a model runs forever while concurrently allowing variation of all model parameters. As output scrolls across the display, the effects of parameter changes are seen immediately. Our strategy is to start with a minimal network model and extend it as forced by the requirement that model behavior mimic observed oscillatory behavior. New learning algorithms were developed for the adjustment of model parameters to match oscillatory behavior, thus allowing prediction of unobserved values. Conversely, if parameter adjustment is impossible, new model currents must be added. The starting point was Hopfield's network model which only includes passive membrane properties and a non-zero, self-recurrent synaptic term, corresponding to a voltage-gated membrane current. Each synapse is modeled by a saturating current. If the product of the self-recurrent synapse's weight with the slope of the linear portion of its current is greater than membrane leak conductance, plateau potentials appear. Addition of a current with a single control parameter which increases from zero results in a Hopf bifurcation to oscillations with non-zero frequency. Each model cell must transduce observed input signal phases to observed output signal phase. For some cells, notably Int1, this requirement forces addition of another current.

465.9

MODELING STUDIES INDICATE THAT SUBTHRESHOLD CURRENTS INTERACT TO SET THE FREQUENCY OF THE NEURAL OSCILLATOR CONTROLLING HEARTBEAT IN THE LEECH.
E. De Schutter* and R.L. Calabrese.
Born Bunge Foundation, University of Antwerp, Antwerp, Belgium and Department of Biology, Emory University, Atlanta, GA 30322

A central neural circuit generates the motor outflow that programs beat timing of the hearts in the medicinal leech. Two pairs of rhythmically active HN interneurons located in the third and fourth segmental ganglia of the ventral nerve cord form the core elements of this neural oscillator; each pair is linked by reciprocal inhibitory synapses and is capable of independent oscillation. Oscillation arises from the interaction of synaptic inhibition and specific ionic conductances. A computer model of one such HN pair was constructed to examine this interaction. It contains equations for fast and slow Ca^{2+} currents, postsynaptic current which depends on presynaptic $[Ca^{2+}]$, the delayed rectifier and an hyperpolorization activated inward current (I_h) derived from voltage clamp studies in the HN neurons. Equations for the fast Na^+ current and A current (I_A) were derived from the literature.

When I_h is blocked in *in vitro* experiments, HN cells stop bursting (Angstadt, J.D. and Calabrese, R.L., J. Neurosci., 9:2846, 1989. This happens in the model but only when a strong A current is present. The actual bursting frequency is determined by the interaction of these two subthreshold currents I_h and I_A.

465.10

GENERATION OF THE SHORTENING MOTOR PATTERN IN THE MEDICINAL LEECH.
G. Wittenberg and W. B. Kristan, Jr. Department of Biology, University of California at San Diego, La Jolla, CA 92093-0322.

Shortening behavior in the leech is on a gross level a simple multisegmental reflex, but detailed analysis of its motor pattern reveals surprising complexities. The gross appearance of the behavior is longitudinal shortening of several segments near the site of mechanical stimulation. However, recordings from motor neurons [MN] innervating longitudinal muscle reveal that the segment closest to the stimulus is bending away from it. A one-sided dorsal stimulus results in excitation of both dorsal excitatory MN and the ipsilateral ventral excitor, and inhibition of the contralateral ventral excitor. A ventral stimulus results in excitation of both ventral excitors and the ipsilateral dorsal excitor, and inhibition of the contralateral dorsal excitor. Inhibitory MN are inhibited ipsilateral to the stimulus, and excited contralaterally. Thus both the inhibitor and excitor MN to the same muscle may be excited, an unexpected finding, but in accord with von Holst's maxim that nervous systems "need the reins just as much as the whip." This is borne out in the responses of MN in the two ganglia anterior and two ganglia posterior to the ganglion in which sensory neurons are stimulated: all longitudinal excitors and inhibitors are excited (with a few exceptions), with the net result the observed longitudinal shortening.

In order to determine the neuronal circuitry responsible for these motor patterns, the connections of several interneurons have been studied. Cell 115, an interneuron involved in both swimming and local bending behavior, plays a role in shortening but excites only dorsal excitors and thus is not sufficient for the whole behavior. Unexpectedly, inhibitory MN contribute to shortening behavior by affecting motor output in adjacent ganglia. For instance, depolarization of the dorsal inhibitory MN causes inhibition of the dorsal excitor in the same ganglion, but causes excitation of the dorsal excitor in the adjacent posterior ganglion. This effect can be explained by the Friesen's finding that the inhibitors make feedback connections to certain swim CPG interneurons, including cell 115. Thus an important role in patterning a "simple" reflex is played by the motor neurons themselves.

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465.11

EFFECTS OF SEROTONIN ON SWIM-GATING NEURONS IN THE MEDICINAL LEECH. J.D. Angstadt and W.O.Friesen. Department of Biology, University of Virginia, Charlottesville, VA 22901.

Prolonged activation of swim-gating cells (e.g. cell 204) is an important step in the initiation of leech swimming behavior (Weeks, J.C. and Kristan, W.B., J. Exp. Biol. 77:71, 1978). Previous studies (Willard, A.L., J. Neurosci. 9:936, 1981) showed that the probability of spontaneous swimming in intact animals or swim-associated electrical activity in isolated nerve cords is increased by the neurotransmitter serotonin (5-HT). We examined the effects of 5-HT on the cellular properties of cell 204 in isolated ganglia (G10-G14). Data from ganglia exposed to 50 μM 5-HT for a minimum of 30 min (n=24) were compared to controls (n=24). Cell 204 was penetrated with a single microelectrode and voltage responses to 1 sec current pulses (-2 to +2 nA) were measured using discontinuous current clamp. We also measured the peak-amplitudes of postinhibitory rebound (PIR) at the offset of hyperpolarizing current pulses and of hyperpolarizing undershoots at the offset of depolarizing current pulses. The I-V curves obtained in the presence of 5-HT were identical to controls except for a small increase in the hyperpolarizing responses to -0.5 and -1.0 nA current pulses. However, in the presence of 5-HT, the peak-amplitude of PIR and of hyperpolarizing undershoots were increased by an average of 190% and 330%, respectively. In Na-free saline, the peak amplitude of PIR was significantly reduced compared to controls. In addition, the serotonin-mediated increase in PIR observed in normal Na saline was eliminated in Na-free saline. These data suggest that ionic conductances intrinsic to swim-gating cells are modulated by 5-HT. Supported by NIH grants NS08089 (JDA) and NS21778 (WOF).

465.12

REAL TIME OPTICAL DETECTION OF RHYTHMIC MOTONEURON AND INTERNEURON ACTIVITY IN THE ISOLATED SPINAL CORD USING DIGITAL IMAGING OF CALCIUM TRANSIENTS. Michael O'Donovan, Wayne Yee & Miklos Antal. Laboratory of Neural Control, NINDS,NIH,Bethesda,MD 20892.

We have used real time digital imaging of calcium transients to identify active motoneurons and interneurons in the lumbosacral spinal cord of E8 to E12 chick embryos during spontaneous, stimulated and NMDA evoked motor activity. The cord was loaded with the calcium indicator Fura-2AM, mounted in a perfusion chamber on the stage of a fluorescence microscope and excited at 340 or 380nm during episodes of motor activity. The fluorescence (F) of loaded cells was monitored with an intensified video camera, and recorded on video tape together with hindlimb muscle nerve activity. At E12, when antagonist motoneurons alternate, the active cells were widely distributed throughout the spinal cord but were concentrated in the lateral motor column (LMC), the intermediate region dorsal to LMC and in the dorsal horn. Fewer active cells were found medial to the LMC. At E8 when the motor pattern is less mature, the active cells were more restricted in their distribution and were concentrated in the LMC and the intermediate region.

During motor activity motoneurons exhibited oscillations of fluorescence ($\Delta F/F = 15$-50%) in phase with rhythmic neural activity recorded from the muscle nerves. Cells in the intermediate region and the dorsal horn, presumed to be interneurons, also showed large fractional changes in fluorescence during motor activity. Some were rhythmically active in phase with motoneurons whereas others were tonically active throughout the episode. In some experiments the calcium transients have been measured in over 50 cells simultaneously. Experiments are now in progress to characterize the active cells histologically.

465.13

NMDA RECEPTORS CONTRIBUTE TO PROLONGED EXCITATION OF THE SCRATCH REFLEX CIRCUIT IN THE TURTLE SPINAL CORD.

Scott N. Currie and Paul S.G. Stein, Department of Biology, Washington University, St. Louis, MO 63130.

Cutaneous stimulation in the turtle produces an increase in the excitability of scratch reflex that outlasts the stimulus by several seconds (J. Neurophysiol. 60:2122, '88). We recently identified "long-afterdischarge" cutaneous interneurons in the midbody spinal cord that may participate in multisecond excitability storage in the scratch reflex pathway (Abstr. Soc. Neurosci. 15:1118, '89). Two characteristics of long-lasting excitation demonstrated for scratch reflex motor patterns are also exhibited by long-afterdischarge interneurons: 1) responses can continue for many seconds after a brief cutaneous stimulus, and 2) strong temporal summation occurs when single electrical pulses are applied to cutaneous afferents at multisecond intervals. In the present study, we tested the ability of D-2-amino-5-phosphonovaleric acid (APV), a specific antagonist of the N-methyl-D-aspartate (NMDA) receptor, to block prolonged cutaneous-evoked excitation. We applied APV to the exposed dorsal surface of a midbody spinal cord segment that receives cutaneous input from the rostral scratch receptive field. We simultaneously recorded single-unit activity from interneurons in the exposed segment and rostral scratch activity from hindlimb motor nerves. 50 μM APV strongly suppressed the activation of long-afterdischarge interneurons and completely blocked the temporal summation of scratch motor output when electrical stimuli were applied to the shell at multisecond intervals; it also greatly attenuated interneuron and scratch responses to higher frequency electrical stimulation and constant-force mechanical stimulation. We conclude that NMDA receptors assist in producing the long-lasting excitation that is a critical component of sensory integration in the scratch reflex pathway. Supported by a postdoctoral fellowship from the Washington University Center for Cellular and Molecular Neurobiology to SNC and NSF Grant BNS-8908144 to PSGS.

CORTEX IV

466.1

TRANSCRANIAL DOPPLER ULTRASONOGRAPHY DURING COGNITIVE STIMULATION. R.E. Kelley, N.J. Tischenkel*, J.Y. Chang*, B.E. Levin*, R.C. Duncan* S-J. Lee*. Dept. of Neurology, University of Miami School of Medicine, Miami, FL 33101

Transcranial Doppler ultrasonography was evaluated for its potential to detect selective cerebral activation during cognitive tasks in 21 normal subjects. Mean and maximum flow velocity of the anterior cerebral arteries (ACAs), middle cerebral arteries (MCAs) and posterior cerebral arteries (PCAs) were measured during performance of a commercial video game with right handed manipulation of the joystick. We also measured flow velocity of the ACAs in 18 subjects during a mental arithmetic task. Serial measurements of the right and left sides were made via a headband with two probes and pCO_2 was monitored throughout the study. We observed global increase in flow velocity above baseline measurements during task performance. During the video game, both MCAs ($t=2.67$, $P=.008$ for the left, $t=4.86$, $P<.001$ for the right) and the left PCA ($t=2.13$, $P=.034$) had selective increase in mean flow velocity compared to the ipsilateral ACA. This selective activation was most prominent in the right MCA where the side to side difference was borderline significant at $F=4.16$, $P=.05$ for mean velocity and $F=3.85$, $P=.06$ for maximum velocity. We did not observe selective activation during the math task. This technique has the potential to allow noninvasive monitoring of circulatory correlates of cognitive activity.

466.2

MOTOR EVOKED POTENTIALS (MEP) INDUCED BY TRANSCRANIAL MOTOR CORTEX STIMULATION: MEP CHARACTERISTICS IN HUMANS WITH REDUCED BRAIN INPUT TO THE SPINAL CORD. M.R. Dimitrijevic, M.A. Lissens*, W.B. McKay. Division of Restorative Neurology and Human Neurobiology, Baylor College of Medicine, Houston, Texas 77030.

We studied motor evoked potentials (MEP's) in 32 subjects with chronic spinal cord injury. MEP's were elicited by transcranial stimulation of the motor cortex and recorded from pairs of surface electrodes placed over the muscles of the lower/upper limbs and trunk. The EMG signals were amplified and recorded by a 16 channel ink-writer and magnetic EMG recorder. The 32 subjects were divided in 5 groups according to the severity of spinal cord lesions.

We found that MEP's were present in subjects with preserved volitional activity and absent in those paralyzed. When analyzing the latency time of MEP's, we found that it was prolonged when compared to healthy subjects. On some occasions, such prolonged latency time could be shortened by conditioning stimuli or suprasegmental maneuvers. However, when volitional activity was well preserved, we also recorded nearly normal MEP latency times.

The results reported suggest that volitional activity is essential for the presence of MEP's and that the characteristics of MEP's depend upon the newly established functional relationship between segmental and suprasegmental structures.

466.3

REPRESENTATION OF MOVEMENTS IN PREMOTOR CORTEX OF SQUIRREL MONKEY: EVIDENCE FOR A HOMOLOG OF THE ARCUATE PREMOTOR AREA. R.J. Nudo. Dept. of Neurobiology & Anatomy, Univ. of Texas Medical School, Houston, TX 77030

We have recently reported that primates possess a unique source of corticospinal fibers, originating within Area 6 in the lateral portions of frontal cortex in prosimians and New World monkeys, and buried in the caudal bank of the inferior arcuate sulcus in Old World monkeys ("Region C"; Nudo & Masterton, 1990). While these results suggest that Region C is common to extant mammals, the functional organization and intracortical connections of this region have been described only in macaques ("arcuate premotor area"; e.g., Muakkassa & Strick, 1979). The present study was undertaken in order to determine whether the functional properties of Region C in squirrel monkey (*Saimiri sciureus*) are similar to those found in the arcuate premotor area in macaque monkey. We employed intracortical microstimulation and retrograde tracing techniques to examine the representation of movements in primary motor (MI) and premotor cortex (Areas 4 and 6, respectively) as well as their intracortical connections. These experiments revealed a distal forelimb representation within premotor cortex of squirrel monkeys, which was well segregated from the more caudo-medial distal forelimb representation within MI. Injection of retrograde tracers into the MI forelimb representation resulted in labeled cells within the Region C forelimb representation. Taken together with our prior descriptions of corticospinal projections from Region C, these results lead further credence to the notion that this cortical region is a homolog of the arcuate premotor area in macaques, and probably emerged in a remote primate ancestor. Supported by NIH NS 27974 and the Whitehall Foundation.

466.4

MICROSTIMULATION MAP OF THE MONKEY PREMOTOR CORTEX. M. Godschalk, A.R. Mitz, J. van der Burg* and B. van Duin*. Department of Anatomy, Erasmus University Rotterdam, POBox 1738, 3000 DR Rotterdam, The Netherlands.

Interpretations of premotor cortex (PM) somatotopy differ between the relevant anatomical (Muakkassa & Strick, Brain Res. 177:176, 1979) and single unit recording (Kurata, Exp. Brain Res. 77:245, 1989) studies. Coarse microstimulation of PM did not resolve this issue (Rizzolatti et al., Exp. Brain Res. 71:491, 1988). A more detailed and complete microstimulation map of the periarcuate and surrounding cortex was necessary.

A large chronic chamber was implanted over the arcuate (AS), superior precentral (SPCS), and central sulci. Biphasic constant-current pulses of up to 65 μA were delivered along 110-180 tracks in each of 4 macaque monkeys.

Movements were evoked from nearly every penetration into PM. Thresholds were typically below 40 μA, and often below 20 μA. Orofacial movements were evoked from the fundus and the caudal bank of the lateral limb of AS. Arm and hand movements were elicited from the caudal bank of AS, along the genu and the adjacent lateral limb. Forelimb sites continued onto the convexity medially and caudally towards SPCS. Thresholds increased with distance from AS caudally. Leg movements were elicited from an area around SPCS. Based upon these results, PM contains a whole body representation nearly parallel to MI, not one that follows the curvature of AS.

466.5

CONTRASTING PROPERTIES OF THREE FRONTAL REGIONS G. di Pellegrino and S. P. Wise Lab. Neurophysiol., NIMH, Poolesville, MD 20837.

Do the reported physiological differences between prefrontal cortex (PF) and frontal motor areas reflect valid contrasts in their properties or do they result instead from the diverse behavioral and physiological methods used to study them? To address this question, a monkey (*Macaca mulatta*) was conditioned to begin each trial by contacting the central of three touch pads. Next, a 1 s-long red or green stimulus appeared directly in front of the monkey, followed by 1.25 s (the first delay period). Then one red and one green light, simultaneously appeared peripherally, each above a potential target. The monkey withheld its response for an *instructed delay period* of 1.25 s, then touched the pad under the peripheral cue matching the first stimulus.

We sampled the dorsolateral, homotypical PF cortex ventral to and within the principal sulcus (97 neurons), the agranular dorsal premotor area (PM, 55 neurons), and a dysgranular region rostral to dorsal PM termed dorsomedial frontal cortex (DMF, 76 neurons). The proportion of movement-related neurons was not significantly different among the three regions explored. However, cells with apparent selectivity for stimulus attributes were found only in PF (10%). Further, during the first delay, phasic responses were prevalent in PF and DMF, but absent from PM. During the instructed delay period, phasic discharges predominated in PF (84%) and DMF (78%), whereas in PM fewer cells (26%) showed this type of activity. Conversely, in PM, tonic discharges were most common (60% *vs.* 9% in PF & 11% in DMF). Onset latency, peak activity latency and activity duration differed between PM and the other two areas. Thus, we found that the previously reported differences between PF and PM are reliable, and do not reflect species, individual, or methodological variation. In addition, the neural properties of DMF and PF more resemble each other than those of PM.

In a task variation, the green stimulus did not appear on trials in which the correct response was to the target beneath the green peripheral light. None of the 6 PM cells tested showed any effect of eliminating the central green stimulus. By contrast, 6 of 9 PF neurons were dramatically affected.

466.6

ACTIVITY OF THE PRECENTRAL MOTOR CORTEX (MI) NEURONS WITH CORTICOCORTICAL AND THALAMOCORTICAL INPUTS. J. Tanji and H. Aizawa., Dept. Physiol., Tohoku Univ. Sch. Med., Sendai, 980, JAPAN

The aim of the present study was to identify cortico-cortical and thalamocortical inputs to MI neurons whose activity properties in relation to a learned motor task were examined. Monkeys (Macaca fuscata) were trained to reach for and push one of two push buttons according to visual instruction signals. When the animal pressed a key for 2 sec, either a right or left push button, placed in a panel facing the animal, was illuminated for 500 ms with an LED from behind. The animal then had to wait for 2.5 to 4.5 sec before releasing the key and push the illuminated button. The neuronal activity in MI was classified into two broad categories, the activity immediately preceding the reaching movement (M) and the activity during the instructed wait-period (I). Under general anesthesia and aseptic conditions, stimulating electrodes were chronically implanted into the supplementary motor area (SMA), postcentral cortex (Post) and thalamus (Thal). MI neurons exhibiting only the I response were frequently activated by SMA but less frequetly by Thal stimulation. Hardly any of them were activated by Post stimulation. Neurons with M responses were most frequently activated by Thal and Post stimulation, and less frequently by SMA stimulation.

466.7

FUNCTIONAL CHARACTERISTICS OF A DIRECT GABAERGIC PATHWAY FROM ZONA INCERTA TO NEOCORTEX IN RODENTS AND PRIMATES. C-S.Lin, M.A.L.Nicolelis, J.K.Chapin and J.H.Kaas. Dept. Physiol., Hahnemann Univ., Phila., PA. 19102, Dept. Psych., Vanderbilt Univ., Nashville, TN 37240

Recently, we have demonstrated a previously unknown but sizable direct GABAergic projection from zona incerta to the neocortex in rats (Science, in press, 1990). This pathway is corticotopically organized and bilateral. To determine whether this pathway is also present in other species, the retrograde fluorescent tracers Fluoro-Gold, Fast Blue, and rhodamine and fluorescein coated microspheres were injected into several cortical areas in the owl monkey. Results of these studies confirmed that this incerto-cortical GABAergic pathway is also present in New World monkeys. Next, to elucidate the functional character of the ZI, single units were recorded in the ZI of rats anesthetized with pentobarbital. Since the trigeminal nuclei are known to project to the ZI, somatosensory mapping studies were carried out. Neurons in the dorsal subregion of the ZI were found to possess very large receptive fields (RFs) on the fur or whiskers of the contralateral or bilateral face. By contrast, neurons in the more ventral subregions of ZI possessed RFs on the ipsilateral face. Both slowly and rapidly adapting responses were found in ZI. In addition, circular visual RFs were found and both on and off cells were identified. Single unit recordings in the general region of ZI in awake, freely behaving rats also revealed neurons with combined somatosensory RFs (whiskers) and visual RFs (covering the visual space in front of the whiskers). These cells were maximally activated during complex coordinated movements of the whiskers when the animal searched the surrounding space or novel objects. Units also responded strongly when an object was rapidly moved toward the eye of the animal. Our present data suggest that ZI may be involved with both sensorimotor integration and attention. *Supported by grants NS26722, AAO6965, KO2-AA00089, AFOSR-90-0266 and FAPES 88/4044-9.*

466.8

FIRING PROPERTIES AND SYNAPTIC RESPONSES HAVE INVERTED LAMINAR DISTRIBUTIONS IN REELER NEOCORTEX. B.W. Connors, L.R. Silva and M.J. Gutnick, Sect. of Neurobiology, Div. of Biology and Medicine, Brown Univ., Providence, RI 02912.

Reeler is a mouse mutation that alters neuronal migration during development, yielding an inversion of the laminar organization of the neocortex. We recorded *in vitro* from slices of normal and reeler parietal cortex to study the influence of laminar position on neuronal membrane properties and synaptic responses. Dye injections in reeler pyramidal neurons revealed atypical morphologies, including distorted apical dendrites and cell inversion. The intrinsic firing patterns, action potential width and height, resting membrane potential, input resistance and evoked EPSPs and IPSPs did not differ between reelers and controls when data were grouped. However, the laminar distribution of intrinsic firing patterns (TINS 13:99, 1990) was inverted in the reeler: intrinsically bursting neurons were found only in layer 5 in the normal mouse, but they were found exclusively in superficial layers of the reeler cortex. The laminar distribution of synaptic responses in the reeler was also inverted: very prominent IPSPs were characteristic of upper layer neurons in the normal mouse, but in the reeler similar responses were observed in deep infragranular layers. We conclude that neurons develop the membrane and synaptic properties appropriate to their function, not their position.

Supported by the NIH, the NIMH and the McCormick Fund.

466.9

LAYER 5 NEURONS CAN INITIATE NMDA-DEPENDENT, 4-7 HZ SYNCHRONIZED RHYTHMS IN NEOCORTEX. L.R. Silva and B.W. Connors, Section of Neurobiology, Div. of Biology & Medicine, Brown University, Providence, RI 02912.

We have studied a cellular mechanism of synchronized rhythms in slices of rat SI neocortex *in vitro*. Slices bathed in nominally zero Mg^{++} showed spontaneous synchronized discharges that were highly rhythmic. Field potentials revealed epochs of 4-7 Hz discharges. Each epoch typically lasted 1-4 sec, and recurred 1-9 times per min for hours. Synchronized rhythms were dependent on NMDA receptors, since they were completely blocked by the specific antagonist AP5. When slices were cut vertically into narrow segments (~1 mm wide), rhythmic spontaneous activity continued in each piece. When segments were then cut horizontally, rhythms continued only in fragments with an intact layer 5. Fragments consisting *only* of layer 5 were also spontaneously rhythmic. Recordings revealed that layer 5 pyramidal neurons whose apical dendrites were severed at the layers 4/5 border had normal firing properties; the majority could oscillate endogenously at 5 to 15 Hz (cf. Silva et al. *Neuro. Abst.* 15:660, 1989). Since layer 5 was necessary and sufficient to produce rhythmic synchronized discharges in this frequency range, we suggest that some EEG rhythms are generated by a network of endogenously oscillating pyramidal neurons in layer 5.

Supported by the NIH, the NIMH, and the McCormick Fund of Stanford University.

466.10

INTRINSIC SUBTHRESHOLD 10-50 HZ MEMBRANE OSCILLATIONS IN INTERNEURONS IN THE FOURTH LAYER OF THE FRONTAL CORTEX.
K. D. Walton, Y. Yarom[1] and R. Llinás, Dept. of Physiology and Biophysics, N.Y.U. Medical Center, 550 First Ave., N.Y., N.Y., 10016 and Dept. of Neurobiology, Hebrew University, Life Sci. Inst. Givat Ram, 91904, Jerusalem, Israel.[1]

Intracellular recordings from interneurons in the layer IV of frontal cortex slices from adult guinea pig generate subthreshold membrane potential oscillations near 40 Hz by the activation of slow Na and K conductances (Llinás and Grace, *Soc. Neurosci. Abst* 15:660,1989. These neurons were identified as belonging to the category of sparsely spinous stellate cells usually considered to be GABAergic interneurons. Further study of the layer IV neurons revealed that, in addition to the 40 Hz oscillatory interneurons, in which an all-or-none plateau potential generates a close-to-fixed frequency oscillation (35 to 46 Hz), a second category of cells exists where the subthreshold oscillation may vary from 10 to 40 Hz. As this second type of cell is depolarized, the oscillatory frequency increases and may reach spike firing level to activate short duration spike bursts at 20 to 40 Hz. We propose that these two types of cells represent two functionally distinct categories of interneurons. In one type a plateau potential, generated by a persistent sodium conductance, may force the cell to oscillate within a narrow frequency range near 40 Hz. The second type of neuron responds with sub-threshold oscillations of variable frequency, depending on the level of membrane depolarization. Both types of neurons are likely to send inhibitory oscillatory input to cortical Pyramidal cells as well as to other neurons within a cortical column. This interneuron network may serve to transform desynchronized thalamic input into 40 Hz oscillatory Pyramidal cell firing, triggered as a rebound response to the synchronized IPSP input from the interneurons. This cortical circuit is proposed to be a part of the cellular mechanism for the "binding" or "conjunction" properties presently considered to be a main function of the 40 Hz rhythm in the brain.

466.11

LONG-TERM RECORDING OF CORTICAL UNITS USING THE CONE ELECTRODE IN MONKEYS. **P.R.Kennedy, R.A.E.Bakay, N.Ovesiku and D.M.Banks*. Georgia Tech and Emory Univ., Atlanta, GA.**

Recording the electrical activity of cortical neurons over many years with adequate signal-to-noise ratios will allow patients to control prosthetic devices. Multi-units, or for more selective control, single units must be recorded for years from voluntary motor areas. From cortical area 4, we have recorded multiple single units for over a year in one monkey, and six months in another. From these signals, we separated single units that were identified over these time periods and related them to movements.

The recording electrode consists of a tiny glass cone with a recording wire fixed inside. Neurites are attracted to grow into the cone by sciatic nerve fibers placed inside at implantation. Details of fabrication, implantation, histology and recording the electrical activity of the ingrown neurites in rat (J. Neurosci. Meth. 29:181-193,1989) are similar in monkey. The multiple units are separated using waveshape matching paradigms (Brainwave Systems Inc., Denver, CO). In one monkey, seven units were identified, of which four were still present at six months when the experiment was terminated. In the other monkey still ongoing at a year, the same three units are identified each month.

The monkeys are videotaped while taking food. On review, the single units are separated using the waveshape parameters determined at earlier sessions. Individual units are reviewed one at a time in synchrony with the videotaped hand and finger movements, thus revealing the relationships between the units and movements.

466.12

TOTAL NUMBER OF NEURONS IN HUMAN NEOCORTEX RELATED TO AGE AND SEX. B. Pakkenberg, S.M. Evans, A. Møller, H. Brændgaard* & H.J.G. Gundersen. Neurological Research Laboratory, Hvidovre University Hospital, Copenhagen and Stereological Research Laboratory, Aarhus University, Denmark.

A method for an unbiased estimation of the total number of neurons in the human neocortex has recently been introduced and applied to 50 brains from normal individuals of different age-groups and both sexes. The sampling was designed so that the majority of cerebral cortex was left intact providing the possibility for resampling and further analysis. Uniform sampling for total neuron number was performed in each neocortical area. Total cortex volume was estimated precisely according to Cavalieri's principle, and neuronal numerical density estimates made in 35 μm thick plastic sections using optical disectors. Normal humans have approximately $20 \cdot 10^9$ neocortical neurons with an inter-individual variation of 15%. There is a decline in the number of neurons with age by 50 to $100 \cdot 10^6$ neurons per year. Females have about 15% fewer neurons than males of same age.

NERVE GROWTH FACTORS IX

467.1

DEPOLARIZATION MODULATES CILIARY GANGLION NEURONAL RESPONSES TO NEUROKINES. F.Fuller, A.Lam*, J.Kloss, B.Cordell, S.Varon† and M.Manthorpe†. California Biotechnology Inc., 2450 Bayshore Pkwy, Mountain View, CA 94043; †Dept. Biology, UCSD, La Jolla, CA 92093.

Neurotrophic agents, including depolarizing concentrations of potassium, prevent the death of cultured chick E8 ciliary ganglion (cCG) neurons. To examine the effects of neurokines independent of their survival activities, we have tested a variety of factors on viable cCG cultures maintained in 40 mM K^+ on a laminin substratum. Without added factors, neurite outgrowth was essentially absent and choline acetyltransferase (ChAT) activity decreased with time. However, IGF-I/Insulin, FGF and CNTF were potent stimulators of both ChAT and neurite outgrowth. FGF elicited the largest increase in ChAT (2 to 3 fold), while CNTF was the most potent (ED_{50} ~5 pg/ml). Under nondepolarizing conditions (4 mM K^+), IGF-I was not active while FGF and CNTF were both able to support survival and neurite outgrowth of cCG neurons and to prevent loss of ChAT activity. Furthermore, CNTF was 10 fold less potent in low potassium compared to ChAT induction in high potassium. These observations suggest that, in vivo, afferent evoked depolarization could similarly modulate neuronal response to neurokines. Supported by NIH 16349.

467.2

REGULATION OF PROTEIN TYROSINE PHOSPHORYLATION IN DEVELOPING MOUSE BRAIN BY EGF, IGF-1 AND INSULIN
Jean-Antoine Girault[1], Gloria Bertuzzi*, James K.T. Wang[2], Dennis Pang* and Paul Greengard Rockefeller University, New York, NY 10021;
[1] INSERM U114, Collège de France, Paris, France;
[2] Tufts University School of Medicine, Boston, MA 02111.

Insulin-like growth factor 1 (IGF-1), insulin, and epidermal growth factor (EGF) have trophic effects on neuronal development *in vitro*. Since their receptors possess tyrosine kinase activity, we have investigated the effects of these growth factors on protein tyrosine phosphorylation in reaggregate cultures from embryonic mouse cerebral cortex, using antiphosphotyrosine antibodies. In the absence of growth factor, two main proteins phosphorylated on tyrosine were observed and designated p120 and p180 on the basis of their apparent molecular weights on SDS-PAGE. EGF enhanced the phosphorylation of p180 and induced the appearance of a 110 kDa and a 170 kDa phosphoprotein, the latter probably corresponding to the EGF receptor. Insulin and IGF-1 increased the phosphorylation of p180 and of a 160 kDa phosphoprotein distinct from the EGF receptor. Platelet derived growth factor, basic fibroblast growth factor, nerve growth factor and bombesin had no effect on protein tyrosine phosphorylation.

In the brains of intact mice, at various ages from embryonic day 16 to the 8th postnatal week, p120 and p180 were the two main proteins phosphorylated on tyrosine observed in basal conditions. The apparent levels of phosphorylation of these 2 proteins increased during development *in vitro* and *in vivo*, reaching a maximum during the postnatal period, earlier for p120 than for p180.

We propose that the phosphoproteins identified in the present study mediate some of the effects of EGF, insulin and IGF-1 in developing central nervous tissue.

467.3

REDISTRIBUTION OF FIBROBLAST GROWTH FACTOR AFTER NEURITIC INJURY Timothy J. Neuberger and George H. De Vries Dept. of Biochemistry, Medical College of Virginia, Richmond VA., 23298

We previously reported the presence of fibroblast growth factor (FGF) in Schwann cells (Sc) and neurons (Nc) in dissociated dorsal root ganglion (dDRG) cultures (Neuberger et. al., 1990). In this study we crushed the neuritic field of established dDRGs and visualized the distribution of FGF at various times after injury. At 1 and 3 days post-injury (DPI), the distribution of FGF appeared unchanged from the normal control culture; FGF immunoreactivity was observed in the cytoplasm of both Nc and Sc but only on the outer membrane of Nc. By 4 DPI, FGF detected on the plasma membrane of the Nc bodies was decreased, whereas FGF associated with the cytoplasm of Nc and Sc remained unaltered. At 4 DPI, the outer membrane of Sc also demonstrated intense FGF immunoreactivity. Most Sc appeared morphologically normal; however, the membrane of a few Sc demonstrated extensive blebbing. At 6 DPI, the FGF distribution was similar to that seen at 4 DPI. In this study, we demonstrate that neuritic injury results in a significant redistribution of FGF; the outer membrane of Sc become positive for FGF while the outer neuronal membrane demonstrates decreased immunoreactivity. The potential role of this redistribution of FGF, in peripheral nerve regeneration is under active investigation. (Supported by NS10821 and NS15408)

467.4

IN VIVO TREATMENT WITH BASIC FIBROBLAST GROWTH FACTOR DURING DEVELOPMENT DOES NOT ALTER NATURALLY OCCURRING NEURONAL DEATH. R. W. Oppenheim, D. Prevette and F. H. Fuller, Dept. of Neurobiology and Anatomy, Bowman Gray School of Medicine, Winston-Salem, NC 27103 and California Biotechnology, Inc., Mountain View, CA 94043

Basic (bFGF) and acidic (aFGF) fibroblast growth factors are polypeptides which act as mitogens for a variety of mesenchymal derivatives. In addition, both aFGF and bFGF promote the in vitro survival of neurons from several different regions of the developing brain and spinal cord and on that basis have been considered as putative neurotrophic factors (NTF). To further explore the role of bFGF as a NTF, we have examined its effects on the survival of several populations of avian neurons in vivo that exhibit naturally occurring cell death. These include: spinal motoneurons, neurons in the ciliary, sympathetic and dorsal root ganglia, and sympathetic preganglionic neurons. Daily treatment of chick embryos in vivo with a wide range of doses of human recombinant bFGF (with and without heparin) during the normal cell death period for the various neuronal types failed to alter the course or magnitude of cell loss. By contrast, in vivo treatment with bFGF was mitogenic for non-neuronal cells as measured by increased numbers of bromodeoxyuridine (BUdR) immunostained cells in the CNS. These data do not support the role of bFGF as a NTF in the survival of avian neurons in vivo.

467.5

BRAIN-DERIVED NEUROTROPHIC FACTOR, BUT NOT NEUROTROPHIN-3 STIMULATES SEPTAL CHOLINERGIC NEURONS IN CULTURE. EFFECTS ON NIGRAL DOPAMINERGIC NEURONS. B. Knüsel, J.W. Winslow, A. Rosenthal, L.E. Burton, D.P. Seid, K. Nikolics and F. Hefti. Andrus Gerontology Center, Univ. of Southern California, Los Angeles, CA 90089 and Genentech, Inc., South San Francisco, CA 94080.

Brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3), two recently cloned molecules closely related to nerve growth factor (NGF), were produced from human cDNA expressed in human embryonic kidney cells. The recombinant proteins were tested in cultures of dissociated fetal rat brain. BDNF but not NT-3 stimulated the differentiation of basal forebrain cholinergic neurons, similarly effective as bFGF (Knüsel, B., et al., *J. Neurosci.*, 10:558, 1990) and as NGF which is well established as neurotrophic factor for these cells. In contrast to NGF and NT-3, BDNF also increased protein content of the cultures. BDNF, as bFGF, was most effective at an early time *in-vitro*. Maximal increase of choline acetyltransferase activity after 3 days treatment with BDNF was approximately 300% of control. Possible actions of BDNF and NT-3 on dopaminergic neurons of ventral mesencephalon in culture are currently studied. Our findings suggest an important role of BDNF in mammalian brain development with a spectrum of actions different from that of NGF.

467.6

FIBROBLAST GROWTH FACTORS IN THE NERVOUS SYSTEM: DISTRIBUTION AND CHANGES AFTER INJURY. F.P. Eckenstein, G.D. Shipley*, R. Nishi. Dept. of Cell Biology & Anatomy, Oregon Health Sci. Univ., Portland OR 97201

Acidic and basic fibroblast growth factors (aFGF and bFGF) are known to stimulate mitogenesis in of a variety of non-neuronal cell types. In addition, recent work has established that FGFs also can act as neurotrophic factors that promote the survival and regeneration *in vitro* of a variety of neurons. The present study addresses the function of aFGF and bFGF *in vivo* by using a mitogenic bioassay on AKR-2B cells coupled with Western blot analysis to quantify levels of aFGF and bFGF in different areas of the rat nervous system. Acidic FGF and bFGF from extracts of nervous tissue were found to differ considerably in their relative dependencies upon heparin to potentiate their mitogenic activities: the effect of aFGF was completely dependent upon heparin whereas the effect of bFGF was only slightly potentiated by heparin. Heparin was also found to stimulate differentially the mitogenic activity of extracts prepared from different areas of the nervous system, indicating that spinal cord, cortex, pituitary, and optic nerve contained different ratios of aFGF to bFGF, whereas sciatic nerve contained extremely high levels of only aFGF. These results were confirmed in Western-blot-experiments, using antibodies specific for either aFGF or bFGF. Transsection of nerves had opposing effects in sciatic and optic nerves: aFGF rapidly declined in the sciatic nerve distal to the cut, whereas FGFs increased slightly in the distal portion of the cut optic nerve. This differential responsiveness of FGF levels in the central versus distal nerve stumps may reflect the differential regenerative potential of these two nerves.

This work was supported by NIH grants AG07424 (FPE), CA42409 (GDS), NS25767 (RN) and a March of Dimes Basil O'Connor grant to FPE.

467.7

CILIARY NEUROTROPHIC FACTOR IN ADULT SCIATIC NERVES IS BIOCHEMICALLY IDENTICAL TO TROPHIC ACTIVITY IN EMBRYONIC CHICK EYES. R. Nishi, T.Holbert* and F.P. Eckenstein. Dept. of Cell Biology & Anatomy, Oregon Health Sci. Univ., Portland, OR 97201

Neurons in the chick ciliary ganglion undergo a marked period of cell death during development that is dependent upon the presence of target tissues in the eye. It has been postulated that the degree of cell death is regulated by neurotrophic factors. Recently, the cDNAs coding for rat (Stöckli et al, 1989. Nature 342: 920) and rabbit (Lin et al, 1989. Science 246: 1023) sciatic nerve ciliary neurotrophic factor (CNTF) were reported; however, because northern blot analysis indicated that the mRNA coding for rat CNTF was not detected in developing tissue, the possibility was raised that the activity of CNTF may be exerted only under "pathophysiological" conditions rather than during neuronal development. We have recentiy (1990, Neuron, Apr) reported the >80,000- fold purification of a growth promoting activity (GPA) from adult chick sciatic nerves that is 50% homologous to rat and rabbit CNTF, thus GPA may be the chicken form of CNTF or a related molecule . In order to test whether this molecule is biochemically identical to a trophic activity previously reported in embryonic chick eyes we ran parallel purifications of chick eye and sciatic nerve material. The chromatographic retention times of the active material from both sources were identical through every step of the purification which included DEAE chromatography, gel filtration, chromatofocusing, and two runs of reverse phase HPLC. In addition, SDS PAGE analysis of the active fractions of both preparations revealed co-migrating 21.5 kD bands that co-purified with the biological activity. Thus, embryonic chick eyes contain a considerable amount of trophic activity for CG neurons that is biochemically indistinguishable from that found in adult chick sciatic nerves, supporting the notion that there is a developmentally relevant function for this molecule. Funded by NS25767 (RN), ALSA (RN), AG07424 (FPE), and March of Dimes (FPE).

467.8

GENE EXPRESSION OF A TROPHIC FACTOR DURING DEVELOPMENT OF EMBRYONIC CHICK CILIARY GANGLION NEURONS. A. Parent, F.P. Eckenstein, and R. Nishi. Dept. of Cell Biology and Anatomy, Oregon Health Sci. Univ., Portland, OR 97201.

It has been postulated that the survival and development of ciliary ganglion (CG) neurons is regulated by neurotrophic factors released by target cells in the eye. Previously, a growth-promoting activity (GPA) was identified in chick eye extracts (Nishi & Berg, 1981), and we have recently reported the >80,000-fold purification and characterization of GPA from chick sciatic nerves (Eckenstein et al., 1990). Amino acid sequence analysis of a proteolytic digestion fragment of GPA shows a 57% homology with a carboxy terminal region of rabbit and rat sciatic nerve CNTF (Lin et al., 1989; Stöckli et al, 1989). The biological activity of GPA and its homology to mammalian CNTF suggests that GPA is the chicken form of CNTF or a related factor. In order to test whether GPA is a neurotrophic factor in vivo for CG neurons we are examining the developmental- and cell- specific expression of the GPA gene. Degenerate oligos were synthesized based on the GPA amino acid sequence and used in a PCR reaction with cDNA synthesized from chick eye and sciatic nerve RNA. Both sources of RNA produced amplification products that hybridized at low stringencies to a rabbit CNTF probe. Thus, the RNA coding for GPA is found in the developmentally relevant source. We expect to use these amplification products as probes to isolate a full-length GPA clone from a cDNA library. The PCR-derived probe will also be used concurrently to analyze GPA gene expression with northern blots. The sequence of the cDNA clone will allow comparison of eye and sciatic nerve GPA with mammalian CNTF. We are especially interested in whether chick eye GPA contains a consensus signal sequence that is lacking in the sciatic nerve GPA. Supported by NS25767 (RN), AG07424 (FPE), March of Dimes (FPE), and the ALS Association (RN).

467.9

FIBROBLAST-DERIVED NERVE GROWTH FACTORS: BIOLOGICAL AND IMMUNOLOGICAL CHARACTERIZATIONS. A. Acheson, P.A. Barker, R. Hodges*, F.D. Miller and R.A. Murphy. Dept. of Anatomy and Cell Biology, Univ. of Alberta, Edmonton, Alberta.

We have investigated the relationship between salivary nerve growth factor (ßNGF) and fibroblast-derived NGF. E10 chick dorsal root ganglia (DRG) contain two subpopulations of neurons, 40% depending on ßNGF for survival and 40% depending on brain-derived neurotrophic factor (BDNF). L929 fibroblast-conditioned medium (LC CM) promotes the survival of 95% of DRG neurons, suggesting that it contains both NGF- and BDNF-like molecules. LC CM also promotes neurite growth from retinal explants, which do not respond to ßNGF, and L cells contain mRNA coding for BDNF. Antibodies to ßNGF completely blocked the survival- and neurite-promoting effects of LC CM, suggesting that molecules with BDNF-like activity are related immunologically to ßNGF. Surfaceplot analyses predict that ßNGF and pig BDNF have similar regions exposed to an aqueous environment and may share common antigenic sites. Antibodies to two synthetic peptides of ßNGF confirm that both NGF- and BDNF-like activities in LC CM arise from molecules that share at least one functionally important epitope. In addition, NGF-like molecules in LC CM can be distinguished immunologically from ßNGF. Thus NGF- and BDNF-like molecules in LC CM may differ from ßNGF and pig brain BDNF, and L cell-derived forms of NGF and BDNF are immunologically related.

467.10

PARTIAL REGIONAL COLOCALIZATION OF HDNF/NT-3 AND BDNF mRNA IN THE RAT BRAIN.

PATRIK ERNFORS, CYNTHIA WETMORE LARS OLSON* AND HÅKAN PERSSON

Department of Medical Chemistry, Laboratory of Molecular Neurobiology, and Department of Histology and Neurobiology, Karolinska Institute, Box 60400, S-104 01 Stockholm, Sweden; and The entry of molecular genetics in the field of neurotrophic factors has recently led to the discovery that nerve growth factor is a member of a family of neurotrophic factors. We have recently isolated and characterized a molecular clone encoding a novel member of this family, named hippocampus-derived neurotrophic factor/neurotrophin-3. We have used *in situ* hybridization to map the expression of both HDNF and BDNF in the rat brain and in the periphery. BDNF mRNA labeled neurons were found in several areas of the brain, whereas HDNF mRNA expressing neruons were restricted to a few regions of the brain. On the contrary, in the periphery cells expressing BDNF mRNA were only found in few tissues. Exept for one structure in the brain stem, HDNF mRNA expression in rat brain was only detected in the hippocampus and developmentally related structures, where it was expressed by neurons in the medial part of CA1, CA2 and dentate gyrus. BDNF mRNA expressing neurons were also seen in the hippocampus, primarily in the hilar region, CA3 and dentate gyrus. Both factors where expressed in a rostro-caudal, dorso-ventral arrangement that were partially overlapping in the hippocampus. In addition, BDNF mRNA expressing neurons were present in the pyramidal layers of cerebral cortex, claustrum and olfactory bulb. In the periphery, HDNF mRNA expressin cells were found in the glomeruli of kidney, in secondary and tertiary follicles in ovaries and in the rat submandibular gland.

467.11

PRIMARY STRUCTURE AND BIOLOGICAL ACTIVITY OF A NOVEL HUMAN NEUROTROPHIC FACTOR NEURONOTROPHIN-3 (NT-3) AND HUMAN BRAIN-DERIVED NEUROTROPHIC FACTOR (BDNF). J.W. Winslow, K. Nikolics, A. Shih*, G.R. Laramee*, T. Nguyen*, D.V. Goeddel*, and A. Rosenthal*. Departments of Molecular Biology and Developmental Biology, Genentech, Inc., South San Francisco, CA 94080.

The structural similarity between NGF and BDNF and their neuronal specificity suggest that additional, functionally distinct members of this protein family may exist. Degenerate oligonucleotides derived from regions conserved between NGF and BDNF were used as primers to amplify human placental DNA by polymerase chain reaction (PCR). Fragments of the expected size (200bp.) were generated and identified as either NGF, BDNF, or a novel related sequence designated human neuronotrophin-3 (hNT-3). A single 1.4kb rat NT-3 clone containing a 258 amino acid open reading frame and two 0.6kb partial hNT-3 cDNAs were isolated from rat and human cDNA libraries. A complete hNT-3 sequence encoding 257 amino acids was obtained by screening a human genomic library while a complete hBDNF 1.2kb clone, encoding a 247 amino acid protein, was obtained from screening a human brain cDNA library. The hNT-3 precursor protein is 44.2% and 38.5% identical to those of the hNGF and hBDNF precursors, respectively, while the putative mature forms shared 57.6% and 55.6% identity. The putative mature forms of human and rat NT-3, and human and porcine BDNF, are identical. Northern blot analysis revealed a broad organ distribution of rat NT-3 poly A+ mRNA including heart, kidney, liver, spleen, lung, and in several brain regions. Rat BDNF mRNA was most prevalent in the brain, however, BDNF mRNA was detected in heart and lung suggesting a role in the peripheral nervous system. Recombinantly expressed and purified hNT-3 was active in the survival of dispersed chick embryonic day-10 sympathetic ganglia (SG) neurons. Purified hNT-3 had potent neurotrophic activity in nodose ganglia (NG), however, only 40-60% of dorsal root ganglia (DRG) neurons survive relative to those in the presence of NGF. Thus, NG and DRG sensory neurons, which innervate mainly visceral and somatic tissues, respectively, may respond to distinct trophic factors because of different target specificities.

467.12

DETECTION OF BRAIN-DERIVED NEUROTROPHIC FACTOR-LIKE (BDNF) BIOLOGICAL ACTIVITY AND mRNA IN SCIATIC NERVE FIBROBLASTS AND SCHWANN CELLS. P. Barker, A. Acheson, S. Pareek, F.D. Miller, and R.A. Murphy. Dept. of Anatomy and Cell Biology, Univ. of Alberta, Edmonton, Alta., Canada T6G 2H7.

BDNF promotes the survival *in vitro* of chick DRG neurons not responsive to NGF, some placode-derived sensory neurons, and retinal ganglion cells. BDNF is produced in the mammalian CNS and may promote neuronal survival centrally in a manner analogous to NGF in peripheral tissues (Nature 341,149, 1989). NGF is produced by non-neuronal cells in the PNS, including fibroblasts and Schwann cells, but cellular sources of BDNF have not been identified.

Our data suggest that BDNF may also be produced by tissues outside the CNS. We have detected BDNF mRNA of sizes 1.7 and 4.0 kb (identical to those in rat brain) in cultured fibroblasts from rat skin and sciatic nerve, in cultured Schwann cells, and in rat sciatic nerve. We also tested the ability of media conditioned by these cells to promote the survival of E10 chick DRG neurons, 40% of which require NGF, and 40% BDNF. Conditioned media (CM) from cultures of dermal fibroblasts and sciatic nerve Schwann cells promoted the survival of 75% and 63% of the neurons respectively, including neurons from both NGF- and BDNF-dependent subpopulations. Chick sympathetic neurons (which require NGF and not BDNF), survived better in NGF-supplemented medium (71% survival) than in CM from fibroblasts (15% survival) or Schwann cells (26% survival). These results suggest that at least two cell types in peripheral tissues that produce NGF may also produce BDNF.

RECEPTOR MODULATION: UP AND DOWN REGULATION II

468.1

LACK OF EFFECT OF CHRONIC ADMINISTRATION OF AGONISTS AND ANTAGONISTS ON RAT CNS RECEPTOR mRNA LEVELS. Z. Zang, M. Riva, H. H. M. Van Tol*†, O. Civelli†-and Ian Creese. Center for Molecular & Behavioral Neuroscience, Rugters, The State University of New Jersey, Newark, NJ 07102, †Vollum Institute for Advanced Biomedical Research, Oregon Health Sciences University, Portland, OR 97201.

Chronic treatments with agonist or antagonist drugs can change the density of CNS receptors. To gain some understanding of the molecular basis of the receptor regulation the present studies examined the mRNA levels for the muscarinic receptors (M_{1-4}) in frontal cortex, hippocampus and brain stem, dopaminergic receptor (D_2) in striatum, as well as the serotonergic receptor ($5\text{-}HT_2$) in striatum and frontal cortex by using Northern blot analyses. Following two to three week treatments via implanted minipumps with scopolamine (10 mg/kg/day) a muscarinic receptor antagonist, haloperidol treatment (0.5 mg/kg/day) a dopamine receptor antagonist, DOI (7.5 mg/kg/day) a serotonergic agonist or ketanserin (7.5 mg/kg/day) a serotonergic antagonist, we found no differences between the control and experimental groups' levels of mRNAs for M_{1-4}, D_2 or $5\text{-}HT_2$ receptors respectively in any brain regions, even though receptor binding showed that these chronic treatments had produced an increase or decrease in the total population of receptors. Within the sensitivity of the assays, these results suggest that the up-regulation of muscarinic receptors and the D_2 dopaminergic receptor by antagonist treatment as well as the down-regulation of the $5\text{-}HT_2$ serotonin receptors by either agonist or antagonist treatments may not be directly regulated at the level of gene transcription.

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468.2

PROTEIN SYNTHESIS IS REQUIRED FOR THE DENERVATION-INDUCED UP-REGULATION OF ACETYLCHOLINE RECEPTOR GENES. Huey-Jen Tsay*, Craig M. Neville*, and Jakob Schmidt. Department of Biochemistry and Cell Biology, State University of New York at Stony Brook, Stony Brook, New York, 11794.

Denervation of skeletal muscle stimulates transcription of acetylcholine receptor genes. To determine if this activation is mediated by the appearance of an activator or the loss of an inhibitor we have investigated the effect of the protein synthesis blocker cycloheximide on the denervation response. White Leghorn chicks (3 d old) were subjected to unilateral section of the sciatic nerve, and cycloheximide treatment (i.p. injections of 0.2 mg/kg at 4-h intervals) was begun. 24 hours later the denervated muscle was assayed by probe excess solution hybridization for acetylcholine receptor α-subunit message and by transcript elongation analysis for α-subunit gene activity. In the presence of cycloheximide the increase in α-subunit message that normally follows denervation was not observed, and α-subunit gene activity dropped to about non-denervated control levels. Block of transcriptional activation was also seen in the case of the receptor δ- and γ-subunit genes. Several nonreceptor genes were not affected by cycloheximide, either in their steady-state levels or in the transcription rates of their mRNAs. During cycloheximide treatment of chronically denervated animals, α-subunit gene transcripts decayed with a half-life of one day. These results suggest that the de novo synthesis of a transcriptional activator is required as a mediating event in the signalling pathway linking the plasma membrane and acetylcholine receptor gene expression.

468.3

DESENSITIZATION OF MUSCARINIC RECEPTORS THAT INHIBIT ADENYLATE CYCLASE IN CEREBELLAR GRANULE CELLS INVOLVES HOMOLOGOUS LOSS OF MUSCARINIC m_2 RECEPTIVE PROTEIN (66K Da) AND HOMOLOGOUS INCREASE IN m_2-mRNA CONTENT. W.J. Wojcik, S. McLeskey, A. Dobrenski and I. Mocchetti§. FGIN and §Anatomy, Cell Biology Dept., Georgetown University, Washington, D.C. 20007.

In primary cultures of cerebellar granule cells, carbachol (carb 100 μM) maximally desensitizes muscarinic receptors that inhibit adenylate cyclase (M-AC). However, muscarinic receptive protein(s) that comprises the M-AC response is unknown and it may be presumptuous to assume the M-AC to be the muscarinic m_2 subtype. Irreversible labeling of muscarinic receptors on membranes prepared from granule cells with ^{3}H-propylbenzilylcholine mustard (PBCM) and separation by SDS-PAGE shows proteins of 92 K Da and 66 K Da in molecular size. In membranes treated with carb (100 μM, 1 hr), ^{3}H-PBCM labels only 92 K Da proteins, suggesting that the desensitized M-AC receptor is a 66 K Da protein. Methoctramine, an antagonist that is 600Xs more potent at M-AC than M-PI, is also shown to prevent the binding of ^{3}H-PBCM to the 66 K Da protein. Desensitization of M-AC involves two events, a short-term process (carb. 100 μM, 1 hr) whereby the M-AC response returns within one hour of resensitization (1 μM atropine, 1 hr) and a long-term process (carb. 100 μM, from 6 to 24 hrs) whereby the M-AC response does not reappear after 1 hr of resensitization. Muscarinic m_2 and m_3 receptor mRNA content was analyzed by Northern blot analysis using probes provided by Dr. T. Bonner, NIMH. Comparisons between Northern blots of poly A$^+$ RNA, that was extracted from cultures exposed to carb (100 μM) for 1, 6 and 24 hours, showed an increased m_2-mRNA content at 6 hrs only and no change in m_3-mRNA. Thus, the M-AC receptor is shown to be the m_2 subtype and desensitization of M-AC results in homologous loss in the 66 K Da protein and homologous increase in m_2-mRNA content.

468.4

A cAMP RESPONSE ELEMENT IN THE β_2-ADRENERGIC RECEPTOR GENE CONFERS TRANSCRIPTIONAL AUTOREGULATION BY cAMP. S.Collins*, J.Altschmied*, J.Bell, M.G.Caron, P.Mellon* and R.Lefkowitz*. HHMI, Duke Univ. Med. Ctr., Durham, NC 27710, Salk Institute La Jolla, CA 92136 and Glaxo Inc., Res. Tri. Pk., NC 27709

The β_2-adrenergic receptor (β_2AR) gene is transcriptionally upregulated in response to agonist or forskolin stimulated cAMP levels. Previous studies show this autoregulation resides in the 5'-flanking region of the β_2AR gene (PNAS, 86:4853,1989). A 34 bp sequence from the β_2AR promoter (-70 to -37), containing the sequence GTACGTCA, confers responsiveness to cAMP in either orientation 5' to a thymidine kinase promoter-CAT reporter gene when transfected into rat C6 glioma or human JEG-3 choriocarcinoma cells. Specific mutations in this sequence completely abolished stimulation. Overexpression of the catalytic unit of protein kinase A fully substituted for the induction of CAT activity by forskolin.

A 43 kD transcription factor (CREB; cAMP response element binding protein) confers cAMP responsiveness through binding to specific sequences. Purified CREB bound to the β_2AR cAMP response element (CRE) in gel-mobility shift assays with an affinity identical to that for the CRE from the human glycoprotein hormone α-subunit gene, and failed to bind to mutated elements.

These results demonstrate an autoregulatory mechanism by which a receptor (β_2AR) stimulatory for adenylyl cyclase exerts positive feedback regulation on its own expression.

468.5

THE ENDOGENOUS NEUROPEPTIDE PEC-60 REDUCES THE AFFINITY OF DOPAMINE D-2 AGONIST BINDING SITES IN RAT NEOSTRIATAL MEMBRANES G. von Euler, S. Ferré*, V. Mutt[1] and K. Fuxe Depts. of Histology and Neurobiology, and [1]Biochemistry, Karolinska Institutet., Box 60400, S-10401 Stockholm, Sweden

Recently, immunoreactivity towards a PEC-60 (peptide with N-terminal glutamic acid, C-terminal cysteine and a total of 60 resudues) antiserum has been demonstrated in all catecholaminergic neurons in the rat brain including dopaminergic nerve terminals in the neostriatum. In order to investigate possible interactions with dopamine D-2 receptors, the effects of PEC-60 were analyzed in vitro on S(-)[N-propyl-^{3}H(N)]-propylnorapomorphine ([^{3}H]NPA; a D-2 agonist in vitro) binding in crude membrane preparations of the rat neostriatum.

PEC-60 was found to produce a concentration-related increase in the K_D value of [^{3}H]NPA binding in rat neostriatal membranes with a maximal increase of 34±18 % at 10-100 nM of PEC-60 (basal K_D values of [^{3}H]NPA binding were 226±15 pM). The number of binding sites were not affected by PEC-60 at these concentrations.

These results indicate the presence of functional PEC-60 receptors in the neostriatum that interacts with dopamine D-2 receptors within the plasma membrane. The present findings may be of relevance for the understanding of D-2 receptor regulation and of D-2 related diseases such as schizophrenia.

468.6

DIFFERENTIAL REGULATION OF [^{3}H] (+)-3-PPP AND [^{3}H] DTG LABELED SIGMA BINDING SITES IN GUINEA PIG BRAIN MEMBRANES BY SUBCHRONIC HALOPERIDOL ADMINISTRATION. W. Karbon and K. Naper*. Nova Pharmaceutical Corporation, Baltimore, MD 21224-2788.

A previous study (Naper, K. et al., *Neurosci. Abstr.*, 1989, 1236) indicated that [^{3}H] (+)-3-PPP and [^{3}H] DTG label pharmacologically distinct sigma binding sites in guinea pig brain membranes, suggesting that these sites might be differentially regulated in vivo. To test this hypothesis, male Hartley guinea pigs were treated for 14 consecutive days with either vehicle or the sigma agent haloperidol (1 mg/kg, i.p.), sacrificed 4 days after the final drug treatment, and brain membranes prepared and assayed for [^{3}H] (+)-3-PPP and [^{3}H] DTG binding. Whereas haloperidol treatment caused a 70% decrease in [^{3}H] (+)-3-PPP binding, only a 15% reduction in [^{3}H] DTG binding was observed. The decrease in [^{3}H] (+)-3-PPP binding most likely reflects a B_{max} change since the affinity of the remaining sites for (+)-3-PPP was unaffected by drug treatment. A significant reduction in [^{3}H] (+)-3-PPP binding, but not [^{3}H] DTG binding, was seen in membranes prepared from guinea pigs sacrificed 28 days after the final drug treatment. 14 day administration of chlorpromazine (30 mg/kg, i.p.) or clozapine (20 mg/kg, i.p.) did not affect sigma binding. These results suggest that [^{3}H] (+)-3-PPP and [^{3}H] DTG labeled binding sites can be differentially regulated in vivo by subchronic haloperidol treatment, an effect which may contribute to the therapeutic efficacy of haloperidol. Furthermore, the findings suggest that haloperidol may not function as a sigma "antagonist" in vivo, since receptor down-regulation is most commonly observed in response to prolonged agonist exposure.

468.7

REGULATION OF TYPE II GLUCOCORTICOID RECEPTOR-LIKE IMMUNOREACTIVITY IN A SUBGROUP OF RAT BRAIN TEL- AND DIENCEPHALIC NEURONS. R.S. Ahima and R.E. Harlan. Dept. of Anatomy, Tulane Univ. Sch. Med., New Orleans, LA 70112.

The rat central nervous system has been mapped for Type II glucocorticoid receptor-like immunoreactivity using monoclonal antibodies. In non-adrenalectomized rats, neuronal immunoreactivity is predominantly nuclear. In most neurons, adrenalectomy abolishes nuclear immunoreactivity and reduces numbers of immunoreactive neurons, while treatment with glucocorticoids increases the intensity of nuclear immunoreactivity as well as numbers of immunoreactive neurons.

Using BUGR2, a monoclonal antibody against the rat liver glucocorticoid receptor, we have localized a subgroup of neurons in CA1 and CA2 of the hippocampus, caudate-putamen, globus pallidus and habenula which show a different pattern of immunoreactivity. Adrenalectomy confers immunoreactivity, while treatment with corticosterone or aldosterone abolishes immunoreactivity. Thus, alterations in intracellular localization of type II glucocorticoid receptor immunoreactivity following adrenal cortical steroid manipulations are dependent on the neuronal type in the brain.

Supported by NIH grant NS24148.

468.8

CHRONIC NALTREXONE TREATMENT INCREASES PREPROENKEPHALIN GENE TRANSCRIPTION. R.S. Roginski, C.M. Knapp and R.S. Zukin. Dept. Neuroscience, Albert Einstein College of Medicine, Bronx, NY 10461.

Long-term blockade of brain opioid receptors by the opiate antagonist naltrexone increases preproenkephalin (PPE) and preprotachykinin (PPT) mRNA in the striatum (Tempel, A., Kessler, J. and Zukin, R.S., *J. Neurosci.*, 10:241-247, 1990). To determine whether transcription of these genes is enhanced, we examined the *in vitro* incorporation of ^{32}P-ribonucleotide triphosphates into nascent RNA in nuclei isolated from rat striatum. Nuclei were isolated from striata of naltrexone-treated or control rats (tissue from 3 brains per treatment group was pooled) and were pulse-labeled with α-^{32}P-UTP for 20 min. Transcription products were quantitated by hybridization on nitrocellulose filters to the cDNAs encoding PPE, PPT, cyclophilin (1B15, a constitutive mRNA) and pSP64 (as the vector control). Autoradiograms were analyzed by computerized densitometry and values for PPE, PPT, 1B15 and pSP64 were determined. Transcription of the PPE and PPT genes were increased by about 128% and 74%, respectively, in the naltrexone group relative to the control group. Since naltrexone increases PPE mRNA 11-fold and PPT mRNA 6-fold, we conclude that a combination of transcriptional and post-transcription processes accounts for the observed upregulation of PPE and PPT gene expression. Moreover, these data support a role for opioid systems in the regulation of PPT gene expression.

469.1

TOWARDS A MODEL OF GERSTMANN-STRÄUSSLER-SCHEINKER SYNDROME IN TRANSGENIC MICE. K. Hsiao*, M.Scott*, D. Foster*, S. J. DeArmond and S. B. Prusiner. Depts. of Neurology, Neuropath., Biochem. and Biophys., Univ. of California, San Francisco, CA 94143.

Gerstmann-Sträussler-Scheinker syndrome (GSS) is an autosomal dominantly inherited human neurodegenerative disease that can be transmitted to non-human primates and rodents through intracerebral inoculation of brain homogenates from patients. Recent studies of GSS demonstrated significant genetic linkage between GSS and a leucine substitution at codon 102 of the human prion protein (PrP) gene (Hsiao, K., et al., *Nature*, 338:342-345, 1989). A founder transgenic mouse, line Tg 174, containing a murine PrP gene with a leucine substitution at codon 101 (homologous to codon 102 in humans) was well until 33 weeks of age when it became lethargic, ataxic, rigid, and paraparetic, rapidly deteriorating over 3 days. The brain of founder Tg 174 showed diffuse spongiform changes and proteinase K-resistant protein reactive with PrP antiserum. Transmission studies of founder Tg 174 brain homogenate to mice are in progress. Two progeny of the Tg 174 founder are well at 19 weeks. Two other founder transgenic mice containing the same PrP gene construct are well at 20 and 26 weeks. If additional Tg mice expressing this PrP mutation develop spontaneous CNS disease, then our results will argue that the clinical and pathological features of GSS may be reproduced in a Tg mouse paradigm. Successful horizontal transmission of disease from Tg 174 would impose new constraints upon models of prion structure.

469.2

GENE TRANSFER OF LACZ TO CNS NEURONS IN ADULT RATS USING REPLICATION-DEFICIENT HERPES VIRUS VECTORS. X.O. Breakefield, E.A. Chiocca, B.B. Choi, W. Cai, N.A. DeLuca, P.A. Schaffer, M. DiFiglia and R.L. Martuza. Depts. of Neurology and Neurosurgery, Mass. General Hosp.; Dana-Farber Cancer Inst.; Neuroscience Prog. and Dept. of Microbiology and Mol. Genetics, Harvard Med. Sch., Boston, MA., 02115.

Our group is developing herpes simplex virus type 1 (HSV-1)-derived vectors that can deliver genes into neurons in the brain without marked toxicity. Three mutant viruses have been tested which possess E. coli lacZ substitutions in the genes encoding the immediate early viral proteins, ICPO and ICP4, and the early protein, thymidine kinase (TK). In all cases lacZ is under viral promoters active early in infection. These mutants are compromised or defective in their ability to replicate, although they can still enter latency. [The TK mutant, RH105, was kindly provided by Drs. D. Ho and E. Mocarski (Stanford Univ.)]. Direct intracerebral inoculation of these vectors (200,000 pfu) into the caudate nucleus and frontal cortex proved relatively nonpathogenic over a 2-4 week period, whereas injection of wild-type KOS virus produced seizures and death within 6 days. The ICPO mutant yielded ß-galactosidase expression in a substantial number of neurons and glia around the inoculation site and at some distance from it for up to 14 days, with evidence of retrograde transport. Inoculations of the ICP4 and TK vectors produced only a few labelled cells in areas immediately adjacent to the injection tract over a few days.

469.3

CIRCLING BEHAVIOR EXHIBITED BY A TRANSGENIC INSERTIONAL MUTANT. A.K.Ratty[a], L.W.Fitzgerald[b], M.Titeler[b], S.D.Glick[b], J.J.Mullins[a*] and K.W.Gross[a]. [a]Dept. of Molecular & Cellular Biology, Roswell Park Cancer Institute, Buffalo, NY 14263, [b]Dept. of Pharmacology & Toxicology, Albany Medical College, Albany, NY 12208.

During the course of making transgenic mice, we found an insertional mutant that expressed an abnormal circling behavior. Mice homozygous for the transgene insertion expressed the circling phenotype, while heterozygous mice did not. The inheritance of the phenotype was consistent with an autosomal recessive made at a single locus. We found that the dopamine D_2 receptor binding sites in pooled striata (both sides combined) of the circling mice were significantly elevated by about 31% compared to striata of normal heterozygous transgenic mice. We found no changes in the levels of several neurotransmitters (dopamine, serotonin, norepinephrine) and their metabolites (dihydroxyphenylacetic acid, homovanillic acid, 5-hydroxyindoleacetic acid) in the striata, nucleus accumbens, frontal cortex and hypothalamus of mutants compared to heterozygous mice. We also did not find evidence of any hearing loss or inner ear deformities or degeneration in our insertional mutant. A DNA fragment from the host genome flanking the 3' end of the integrated transgene was found to map to mouse chromosome 16 by analysis of the strain distribution patterns of RFLPs in recombinant inbred strains. The transgene integration in the TgX15 mouse line disrupted an endogenetic locus affecting motor function. This is the first instance in which insertional mutagenesis has resulted in a well-characterized behavioral abnormality.

469.4

PROFILE OF MESENCEPHALIC DOPAMINE NEURON LOSS IN WEAVER MUTANT MICE DURING LIFE-SPAN. B. Ghetti and L.C. Triarhou. Department of Pathology (Neuropathology) & Program in Medical Neurobiology, Indiana University School of Medicine, Indianapolis, IN 46202

Weaver mutant mice (wv/wv) live up to 24-30 months on the B6CBA-A^{w-J}/A hybrid stock; loss of midbrain dopamine (DA) neurons by P20 and P90 is part of their phenotype (Exp. Brain Res. 70: 256-265, 1988). In the present study we (1) investigated whether midbrain DA cell loss occurs in wild-type (+/+) B6CBA-A^{w-J}/A mice with age, and (2) extended the information on DA cell loss in weaver mutants by obtaining a profile throughout their life-span. Counts of TyrOHase immunoreactive neurons did not reveal loss in any of the mesencephalic DA cell groups (A8, A9, A10) of +/+ animals at 18 (n=2) or 30 (n=2) months. The average number of neurons (± SEM) from +/+ mice of all ages (n=10) was 1270 ± 42 DA cells in A8, 3951 ± 292 in A9 and 3786 ± 126 in A10. Weaver mutants were studied at 20 days (n=3), 3 (n=3), 12 (n=3), 18 (n=1), and 24 (n=3) months. During the first year of life, the maximum loss, amounting to 50% in A8, 70% in A9 and 20% in A10, is already observed at 3 months. At 2 years of age, losses are severer and amount to about 60% in A8, 85% in A9 and 35% in A10. These findings indicate that there is a second wave of DA cell loss in weaver mice, taking place in an older age but at a slower rate; it remains to be established whether such a loss represents an accelerated form of aging of genetically susceptible neurons or an effect of the mutation irrespective of aging phenomena. (Supported by PHS RO1-NS14426).

469.5

DOPAMINE CONTENT, TYROSINE HYDROXYLASE ACTIVITY, AND DOPAMINE UPTAKE IN THE STRIATUM OF THE WEAVER MUTANT MOUSE. E.H. Stotz, J.R. Simon, B. Ghetti. Prog. Med. Neurobiol., Indiana Univ. Sch. of Med., Indpls., IN 46202.

Compared to normal (+/+) mice, the weaver mutant mouse (wv/wv) has decreased dopaminergic input to the striatum from the substantia nigra (SN). In 5 pairs of 3 month old +/+ and wv/wv mice, dopamine (DA) levels and tyrosine hydroxylase (TH) activity were determined by HPLC-EC, and DA uptake was measured in sucrose homogenates from the same striatal samples. DA levels and TH activity in the wv/wv mice were decreased approximately 60% relative to +/+ controls. Striatal DA uptake in the wv/wv was decreased by 95%, thus being more severely affected than either DA content or enzyme activity. The present neurochemical data on DA content and TH activity are in agreement with previous morphological data on cell loss in the SN. Since DA uptake is more severely affected than the aforementioned parameters, it appears that the remaining DA neurons in the wv/wv striatum are functionally inadequate.

Kinetic analyses of TH was carried out in other mice and indicated that the wv/wv enzyme had a higher Km and a lower Vmax than control. While neither the changes in Km nor Vmax alone seem to account for the observed reduction in TH activity in the wv/wv, such reductions may be attributed to the combined kinetic shifts. (RO1 NS 14426)

469.6

ALTERED DEVELOPMENTAL AND TISSUE SPECIFIC REGULATION OF GENE EXPRESSION IN MOUSE TRISOMY 16, A MODEL OF DOWN SYNDROME. D. M. Holtzman, R. M. Bayney*, C. N. Berger*, C. J. Epstein*, and W. C. Mobley. Depts. of Neurology, Pediatrics, and Biochemistry, UCSF, San Francisco, CA 94143; Molecular Therapeutics Inc., West Haven, CT 06516

Our goal is to understand better how mammalian aneuploidy affects gene expression. Mouse trisomy 16 (Ts 16), an animal model for Down syndrome, provides an opportunity to study the regulation of gene expression in cells with a 50% increase in normal gene dosage. We have examined expression of the gene encoding the amyloid precursor protein (APP), located on mouse chromosome 16 and human chromosome 21, in the brain and other organs of fetal Ts 16 mice and adult Ts 16/2N chimeras. Although a 1.5 fold increase in APP mRNA levels per trisomic cell might be expected, there are examples in human Ts 21 and mouse Ts16 of greater increases in brain. APP has 3 RNA transcripts which code for proteins 770, 751, and 695 amino acids in length. We used northern blot analysis and the polymerase chain reaction to quantitate total APP mRNA as well as the mRNA transcripts encoding APP 695, 751 and 770. In all organs examined, total APP mRNA expression was increased by at least 2 fold in Ts 16 versus controls; however, the increases were both developmentally regulated and tissue dependent. APP 695 mRNA was found in very low levels in normal fetal lung whereas this transcript was of greater or equal abundance than both other APP transcripts in Ts 16 fetal lung. All 3 APP transcripts were increased 2 fold in fetal Ts 16 whole brain versus control littermates. Interestingly, adult Ts 16/2N chimera brain, which was 40-50% Ts 16, showed approximately a 5 fold increase in all APP transcripts versus controls. This increase is 9 times more than expected if a strict gene dosage effect were operative as has been observed for other loci. Although the mechanism of these changes is unclear, our results with this gene suggest that an increase in gene dosage may initiate a complex change in gene expression which is tissue specific, developmentally regulated, and likely to be important in the pathogenesis of a number of abnormalities found in the nervous system and other organs in aneuploid conditions.

469.7

^{99m}TECHNETIUM AUTORADIOGRAPHIC ANALYSIS OF BRAIN LESIONS IN T-CELL RECEPTOR TRANSGENIC AUTOIMMUNE MICE.

JM Mountz, JD Mountz*, PS Sherman*, PE McKeever, JM Rowe*. Univ. of Michigan, Ann Arbor, MI 48109 and Univ. of Alabama, Birmingham, AL.

We have established that MRL-*lpr/lpr* (MRL-*l*) systemic lupus erythematosis (SLE) mice show multiple regions of decreased tracer uptake in the central nervous system (CNS) on ^{99m}Tc Hexamethylpropyleneamine Oxime(HMPAO) autoradiography which can be attributed to loss of blood brain barrier (BBB) integrity. These autoradiographic defects are not seen in MRL+/+ normal controls. The histopathologic nature of these defects are unlike other organ specific lesions in that they do not show characteristic perivascular cuffing, and thus may be mediated by autoantibody production. To determine if auto-reactive T cells were involved, experiments utilizing MRL-*l* mice backcrossed to T-cell receptor (TCR) transgenic (TR) mice were performed. In TCR-TR mice, most thymocytes are reactive with H-2D^{b} and the male H-Y antigen. The mouse brains were prepared for autoradiography after an I.V. injection of ~4 mCi of ^{99m}Tc HMPAO in 0.1 ml of sterile saline. These and other brains were also stained with hematoxylin and eosin. Serial 20 micron thick sections showed no identifiable characteristic defects in the TCR-TR MRL-*l* mice as was observed in non TR MRL-*l* mice. Using the horseradish peroxidase (HRP) method to evaluate BBB integrity, there was no evidence of parenchymal infiltration of HRP as seen in MRL-*l* mice. In conclusion, the presence of the T-cell receptor transgene eliminated the characteristic defects seen in MRL-*l* mice. This suggest that a normal T-cell receptor repertoire is necessary for development of the lesions, but direct interaction of T cells with the brain might not be required. We propose a two step model for development of the CNS defects: 1) antigen specific T cell autoreactivity with endothelial antigens distant from but cross reactive with BBB endothelium and 2) the production of autoantibodies which cross react with BBB endothelium. This model supports the use of medical therapy that enhances BBB integrity rather that those that suppress direct lymphocyte mediated tissue inflammation in the treatment of CNS disease of SLE.

469.8

COMPLEX NEURAL SYSTEMS AND BEHAVIORS: A NOVEL STRATEGY FOR THEIR GENETIC DISSECTION.

C. Vadasz[1,2], I.Laszlovszky[1], P.Kabai[1*], M.Sasvari[1*], I.Sziraki[1*], I. Vadasz[1*], A.Lajtha[1,2]. [1]Neurochem. Div., N.S.Kline Inst. for Psychiatric Res.,Orangeburg,N.Y.,10962 & [2]Dept. of Psychiatry, New York University Medical Center, 550 First Avenue, New York, N.Y. 10016

Most aspects of brain function are complex and genetically variable. To eliminate major obstacles in the genetic analysis of catecholamine neurotransmitter mechanisms, we initiated a study to transfer genes that influence the activity of mesencephalic tyrosine hydroxylase (TH/MES), the rate limiting enzyme in catecholamine biosynthesis, to the same genetic background. The transfer of genes was carried out by backcross-intercross cycles with concomitant selection for TH/MES. We have succesfully completed five cycles establishing replicated high (B6.C alpha and beta) and low (B6.I alpha and beta) genetically standardized stocks. The B6.C and B6.I populations evince a highly significant difference in TH/MES, while at cycle M_5 the probability of incrosses at any nonlinked, nonselected locus is 0.948. Development of sets of congenic recombinant inbred, neurological animal model lines with different mesencephalic dopamine systems provides an analytical tool for mechanism-oriented experimentation.

469.9

SYNAPTOSOME UPTAKE OF [3H] NOREPINEPHRINE, [3H] DOPAMINE and [3H] SEROTONIN IN CANINE NARCOLEPSY

Delphine Valtier, William C Dement and Emmanuel Mignot*. Sleep Research Center, Stanford University School of Medicine, Palo Alto CA 94304 (USA).

Canine narcolepsy, a model of the human REM sleep disorder, is associated with altered catecholamine metabolisms in various brain areas. A possible explanation for these global changes could be the existence of specific defects in monoamine uptake processes. In order to investigate this hypothesis, we have studied the uptake of [^{3}H] norepinephrine (NE), [^{3}H] dopamine (DA) and [^{3}H] serotonin (5-HT) in synaptosomes prepared from slowly frozen brain cortical tissues (Dodd, Neurochem Path 4: 177-198.1986) in six narcoleptic (N) and six control (C) doberman pinshers (age and sex matched). The effect of the freezing protocol has been tested and no differences were found between fresh and frozen maximal velocity (Vmax) or affinity (Km) for the transporter. All subsequent experiments were then carried out using frozen tissues. Significant uptake processes for [^{3}H] NE, [^{3}H] DA and [^{3}H] 5-HT were found in all control and narcoleptic dog cortical brain samples studied. No signiticative differences in Vmax or Km were observed between narcoleptic and control animals. Noticably, [^{3}H] Norepinephrine Vmax and Km were found to be higher in both fresh and frozen samples of the control dogs. Further animals will be studied to confirm this observation and the same protocol will be applied to the amygdala, a structure were monoaminegic abnormalities have been well characterized in narcoleptic dogs. Research supported by NS 23724-03.

469.10

NEW INBRED RAT STRAINS: WK-HT WITH GENETIC HYPERTENSION, AND WK-HA WITH GENETIC HYPERACTIVITY.

E.D.Hendley and W.G.Ohlsson*. Univ. of Vermont, Burlington VT, 05405.

The spontaneously hypertensive rat (SHR), and normotensive, Wistar-Kyoto (WKY) controls,are widely employed in studies of essential human hypertension. SHR are also behaviorally hyperactive, and this led us to use genetic means to separate the hyperactivity trait from the hypertensive trait a decade ago. We recombined the genes of SHR and WKY by crossbreeding, then selected brother/sister pairs in the F2 and successive generations to produce two new strains, now nearly fully homozygous. WK-HTs are hypertensive but normoactive, and WK-HAs are hyperactive but normotensive. Since SHR have both traits and WKY have neither, we use all four inbred strains to seek correlations of biological differences with one or the other of both traits. Longitudinal studies revealed that hypertension persists throughout at least two years in SHR and WK-HTs, and similarly, hyperactivity persists in SHR and WK-HAs for at least two years. Studies in the four strains also revealed that not all behaviors of the SHR are present in WK-HAs, and not all cardiovascular changes in SHR are associated with hypertension. We suggest that WK-HTs are an improvement over the SHR as a model of hypertension, and that WK-HAs provide a unique animal model of hyperactivity. Supported by NIH RO1-NS26390, and NSF R11-860679.

469.11

GENES THAT CAN MUTATE TO INDUCE NEURONAL DEGENERATION IN *C. ELEGANS*.

M. Driscoll* and M. Chalfie. Dept. of Biological Sciences, Columbia University, New York, NY 10027

Rare dominant alleles of two genes, *mec-4* and *deg-1*, produce abnormal, toxic products that result in the vacuolar degeneration of small groups of neurons in the nematode *Caenorhabditis elegans*. Three dominant alleles of *mec-4* induce degeneration of six touch receptor neurons. In order to understand the molecular causes of degeneration, the *mec-4* gene has been cloned and sequenced. *mec-4* appears to be a membrane protein that does not show extensive homology with current GenBank entries. The amino acid changes in the degeneration-inducing *mec-4* variants have been determined. Studies of the molecular requirements for degeneration will be discussed.

The *mec-4* gene and the *deg-1* gene are members of a *C. elegans* gene family. Molecular analysis has demonstrated that the encoded proteins are 50% identical over a large portion of their legnths. We call the proteins encoded by this family of genes "degenerins". Homology relationships among degenerins will be discussed.

470.1

CELLULAR LOCALIZATION OF 5-HT$_{1A}$ RECEPTOR mRNA IN THE RAT BRAIN. J.M.Palacios, M.Pompeiano* and G.Mengod. Preclinical Research, Sandoz Pharma Ltd., CH-4002 Basel.

Different oligonucleotides from the coding region of the rat 5-HT$_{1A}$ receptor gene were used to examine the distribution of cells containing transcripts for this receptor in the rat brain by using in situ hybridization histochemistry. The specificity of the hybridization signals was verified in control experiments and Northern analysis. The results obtained revealed that the presence of transcripts was abundant in the hippocampus, lower in the midbrain and cortex, and absent in the cerebellum. The highest levels of hybridization signal were seen in the hippocampus, entorhinal cortex, septum, nucleus of the vertical limb of the diagonal band, interpeduncular nucleus, all raphe nuclei, ventral nucleus of the lateral lemniscus, olfactory bulb and cerebral cortex. This distribution was in very good agreement with that of receptor binding sites labeled with [^{3}H]8-OH-DPAT. These results demonstrate that different neuronal populations express the 5HT$_{1A}$ receptor mRNA. They also suggest that the same gene codes for presynaptic autoreceptors in the raphe, presynaptic heteroreceptors in the cholinergic cells of the septum and postsynaptic receptors in the hippocampus.

470.2

IN VIVO STUDIES OF σ RECEPTORS WITH RADIOLABELED HALOPERIDOL DF Wong, R Gibson†*, ED London, HD Burns†*, E Shaya*, RF Dannals*, HT Ravert*, AA Wilson*, HN Wagner Jr.* Johns Hopkins Medical Inst., Baltimore, MD 21205 and Merck (MSD Res. Labs) West Point PA† 19486.

We have assessed the use of [^{18}F]haloperidol (FHAL) for imaging and quantification of brain σ receptors in vivo. FHAL uptake in striatum (ST), cerebellum (Cb), and cortex (Cx) peaked at 30 min post-injection (~8% injected dose/g tissue); a slow dissociation was observed. Competition with IV d-pentazocine (d-PENT, 25 mg/kg) produced 15-30% inhibition in several brain regions. Studies with IV SKF 10,047 (d-NANM; 25 mg/kg) produced 23% inhibition in Cb. As FHAL binds to dopamine as well as σ-receptors, we tested the effect of spiperone on FHAL uptake in ST. Spiperone (0.5 mg/kg IP) inhibited FHAL binding by 27%. However, both d-PENT and d-NANM increased FHAL binding by 30-40%. These results suggest that d-PENT and d-NANM may decrease synoptic dopamine, which would compete with FHAL for D_2 dopamine receptor. We have confirmed these results using [^{3}H]haloperidol (HHAL). HHAL binding in ST increased 60-70% with d-PENT 5 mg/kg and d-NANM 25 mg/kg and declined by 57% in the presence of d-amphetamine 7 mg/kg.

These studies support development of radiolabeled haloperidol as an in vivo ligand for σ receptors, and support an action of σ ligands to decrease endogenous dopamine.

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470.3

EXPRESSION OF THREE ALPHA-2 ADRENERGIC RECEPTOR SUBTYPES IN RAT TISSUES: IMPLICATIONS FOR ALPHA-2 RECEPTOR CLASSIFICATION. W. Lorenz*, J.W. Lomasney*, S. Collins*, A.L. Sylvia, M.G. Caron and R.J. Lefkowitz*, H.H.M.I. and Depts. of Medicine and Cell Biology, Duke University Medical Center, Durham, NC 27710.

Based on ligand binding studies in various tissues and species, evidence for several α_2-adrenergic receptor subtypes has accumulated. Currently the α_2-adrenergic receptors are classified exclusively by pharmacological criteria. The molecular cloning of three distinct genes for human α_2-adrenergic receptors confirmed the demand for multiple α_2-adrenergic receptor subtypes. According to their localization on human chromosomes the genes were termed α_2-C10, α_2-C4, and α_2-C2. The relationship between the pharmacological classification of α_2-receptors and the isolated genes has yet to be clarified. Using northern blot hybridization we analyzed the expression of the three cloned α_2-adrenergic receptor genes in 13 rat tissues, and in cell lines previously described as model systems for pharmacologically defined α_2-adrenergic receptor subtypes. The α_2-C10 receptor corresponds to the α_{2A} subtype and is expressed in rat brainstem, cerebral cortex, hippocampus, pituitary gland, cerebellum, kidney, aorta, skeletal muscle, spleen and lung. Messenger RNA coding for the α_2-C4 receptor was detected only in brain regions, not in peripheral tissues, whereas the α_2-C2 message was found only in liver and kidney. Hybridization experiments with RNA from model systems which formed the basis for pharmacological α_2-receptor classification lead to the conclusion that the pharmacological subtype α_{2B} represents two distinct receptor molecules; the α_2-C4 and a subtype previously undetected by classical ligand binding approaches. Furthermore, our experiments suggest that the pharmacological subtype α_{2C} is an inter-species variation of α_2-C4 rather than a separate subtype. Finally, the α_{2B}-like receptor α_2-C2 was found not to be covered by the current pharmacological classification.

470.4

AUTORADIOGRAPHIC LOCALIZATION OF PUTATIVE SIGMA RECEPTORS IN THE PRIMATE AND HUMAN BRAIN. A.E. Ciarleglio and D. C. Mash, Departments of Neurology and Pharmacology, University of Miami School of Medicine, Miami, FL. 33101

Sigma receptors are the current target for antipsychotic drug development. Novel antipsychotic agents which possess selective and high affinity for the putative sigma binding site have demonstrated beneficial effects in schizophrenic patients in recent clinical trials. These agents may serve as an alternative to the principal neuroleptic drugs currently in clinical use which mediate extrapyramidal side effects and dyskinesias through their blockade of dopamine receptors. We have used *in vitro* autoradiography to localize putative sigma receptors labelled with (+)-[^{3}H]-3-(3-hydroxyphenyl)-*N*-(1-propyl) piperidine ([^{3}H]-PPP) in the primate and human brain. The binding characteristics of [^{3}H]-PPP in the primate brain were comparable to those previously described in the rodent. Saturation analysis demonstrated a single class of sites in cerebellar and hippocampal membranes (K_D = 10-20 nM). Computer-assisted densitometry demonstrated that all paralimbic and limbic regions including the amygdala, hippocampus, orbitofrontal, cingulate, insular, and temporopolar areas displayed peak densities of [3H]-PPP binding. Moderate labelling of sigma receptors was seen throughout the hypothalamus. A striking enrichment of binding was apparent over the suprachiasmatic and supraoptic nuclei. Moderate to low levels of sigma binding sites were observed over the ventromedial soctors of the caudate and the putamen. Within the brainstem, sigma receptors were elevated over the cerebellar vermis. Taken together, these observations suggest an association of sigma receptors with the limbic system. The targeting of novel sigma-selective agents to limbic brain areas may explain, in part, the beneficial effects of these drugs in schizophrenia. Supported by DA06227.

470.5

PARTIAL OVERLAP IN THE DISTRIBUTION OF MONOAMINE OXIDASE TYPE A (MAO-A) AND SIGMA RECEPTORS IN RAT AND MOUSE BRAIN. M. Basile, D. C. Mash, and Y. Itzhak, Depts. Neurology, Pharmacology, Biochemistry and Molecular Biology, University of Miami School of Medicine, Miami, FL. 33101.

Biogenic amines are metabolized by monoamine oxidase. Selective inhibitors of MAO type A (i.e., clorgyline) exhibit high affinity for sigma binding sites (Eur. J. Pharmacol. 176: 107, 1990). The present study was undertaken to examine the distribution patterns for [^{3}H]-MPTP binding to MAO-A and [^{3}H]-3-PPP binding to putative sigma receptors. MAO-A sites were labelled in rat and mouse brain with [^{3}H]-MPTP in the presence of sufficient deprenyl to occlude [^{3}H]-MPTP binding to MAO-B. Adjacent, slide mounted sections were labelled with 8 nM (+) [^{3}H]-3-PPP to visualize putative sigma receptors. Sigma receptor densities were elevated in the superficial layers of the cerebral cortex, the dentate gyrus of the hippocampus, the hypothalamus, the locus coeruleus and the cerebellum. Moderate [^{3}H]-3-PPP binding was observed in the caudate-putamen and in parts of the thalamus. [^{3}H]-MPTP binding sites associated with MAO-A were dense in the ventral sector of the striatum, the dentate gyrus of the hippocampus, the hypothalamus and the locus coeruleus. Very low levels of [^{3}H]-MPTP binding were detected over the cerebellum and cerebral cortex. These observations demonstrate a partial overlap in the distribution of MAO-A and sigma binding sites. Taken together, the in vitro binding and autoradiographic studies provide support for the possible existence of sigma receptor subtypes. One subtype of sigma binding site may be associated with the enzyme, MAO-A. Supported by a grant from the NPF, Miami, FL. 33101.

470.6

MUSCARINIC BINDING SITES IN THE DORSOMEDIAL MEDULLA: AUTORADIOGRAPHIC AND BIOCHEMICAL STUDIES. P. Ernsberger, Depts. of Medicine & Neuroscience, Case Western Reserve Univ. School of Medicine, Cleveland, OH 44106.

Structures in the dorsomedial medulla (DMM) mediate cardiorespiratory control. Muscarinic receptors participate in central autonomic regulation. In this study, muscarinic receptors labeled with [^{3}H]QNB were characterized in rat brain sections and cow DMM membranes. Quantitative image analysis of autoradiograms from rat medulla sections (15 μm) labeled with [^{3}H]QNB (1 nM in Krebs', 60 min at 22°C) revealed that muscarinic binding (fmol/mg tissue, mean±SE of 15-66 determinations) was heterogeneously distributed in the hypoglossal complex (*m*: 316±8, *vl*: 267±4, *d*: 299±3, *n Rotter*: 101±4) and the NTS region (*vl*: 136±3, *v*: 126±3, *m*: 142±4, *dmnv*: 290±6, *dl*: 140±3, *ni*: 122±4, *nI*: 149±3, *AP*: 52±3, *C2 area*: 147±6, *n parasolitarius*: 89±3). Outside the DMM, muscarinic binding was abundant in the bed nucleus of the transtegmental tract (112±2) but not the parapyramidal area (54±2). Membrane binding assays with cow DMM showed a high density (B_{max} = 231±10 fmol/mg protein) of high-affinity (K_d = 0.46±0.05 nM) [^{3}H]QNB binding sites. Muscarinic antagonists competed for [^{3}H]QNB binding with the following order of potency (K_i in nM): scopolamine (1.6±0.3) > 4-DAMP (2.9±0.3) > atropine (5.1±0.7) >> pirenzepine (110±6) = methoctramine (129±9), a profile consisitent with the M3 subtype. Hill slopes were close to one, indicating a single population of sites. For agonists, the order of potency was: oxotremorine (102±8) > pilocarpine (1100±120) > methacholine (8100±830) > McN-A-343 (16,000±1700), also consistent with the M3 subtype. In DMM, muscarinic binding sites are present in structures with cardiorespiratory function and appear to be exclusively of the M3 subtype.

470.7

PHOSPHOINOSITIDE TURNOVER: AUTORADIOGRAPHIC IMAGING IN THE BRAIN DIFFERENTIATES GLUTAMATERGIC AND CHOLINERGIC SYNAPTIC RESPONSES. P.M. Hwang, D.S. Bredt and S.H. Snyder. Johns Hopkins Univ. Sch. of Med., Dept. of Neurosci. Baltimore, MD 21205

The phosphoinositide (PI) second messenger system mediates numerous neurotransmitter effects in the brain, which, with some exceptions, have not been readily assigned to specific cellular sites. Localization of neurotransmitter synaptic responses in the brain has been explored by autoradiographic mapping of receptor binding sites, but these sites sometimes do not reflect known synaptic input. Ideally, one would like to image functional, second messenger responses to neurotransmitters at specific loci in the brain. However, monitoring the generation of [^{3}H]inositol phosphates in response to neurotransmitter agonists is not compatible with anatomical localization. Recently, Godfrey (Biochem. J. (1989) 258, 621) measured PI turnover in brain slices with [^{3}H]cytidine as a precursor. In this technique the generation of [^{3}H]cytidine diphosphate diacylglycerol ([^{3}H]CDP-DAG) reflects PI turnover. Since CDP-DAG is membrane bound, we attempted to localize [^{3}H]CDP-DAG by autoradiography, rinsing away water soluble metabolites. Using [^{3}H]cytidine as a precursor, we report discrete localizations of phosphoinositide turnover in brain slices and peripheral tissue by selective autoradiography of [^{3}H]CDP-DAG.

470.8

AUTORADIOGRAPHIC LOCALIZATION OF FLUNITRAZEPAM BINDING SITES IN DIAZEPAM-SENSITIVE AND -RESISTANT MICE. E.J. Gallaher, S.E. Gionet*, J.K.Belknap. VA Medical Center and Depts. of Pharmacology and Medical Psychology, Oregon Health Sciences Univ., Portland, OR 97201.

Diazepam-sensitive (DS) and -resistant (DR) mice were developed by selective breeding based on the duration of impairment on the rotarod following a standard dose of diazepam. Initial studies of [3H]-flunitrazepam (FLU) binding failed to indicate differences between DS and DR mice in either whole brain or in dissected brain areas (hippocampus, cortex, cerebellum). However, significant differences in small anatomical areas would not be observed using this method. We therefore initiated an autoradiographic study to survey the density and location of various GABA-benzodiazepine receptor ligands throughout the mouse brain. In the current study we report the distribution of [3H]-FLU binding throughout the brain of DS and DR mice.

Brains were sliced into16-micron sagittal frozen slices, thaw-mounted on slides, and stored frozen until incubation with ligand. Slices from paired DS and DR mice were incubated simultaneously with [3H]-FLU, and were then exposed to [3H]-sensitive film for seven days. Images were photographed with a video camera, digitized, stored on a hard disk drive, and analyzed with a MicroComp DS microdensitometry system. Brain areas were delineated and receptor densities were determined after subtraction of background density.

We quantified receptor density in whole brain, cerebellum, cortex, hippocampus, hypothalamus, thalamus, corpus striatum, superior and inferior colliculi, and substantia nigra. Our initial analyses suggest that differences between DS and DR mice are either small or insignificant, consistent with the earlier studies. These findings indicate that behavioral differences observed in DS and DR mice are not a result of differences in receptor number, suggesting that DS/DR behavior is a result of alterations in receptor subunit structure and function.

Supported by PHS Grant NS23927 and the VA Medical Research Service.

470.9

PERIPHERAL BENZODIAZEPINE RECEPTOR SITES IN HUMAN BRAIN GLIOMAS. M. Diksic, A. Takada and Y.L Yamamoto. Montreal Neurological Institute, McGill University, Montreal, CANADA, H3A 2B4

Peripheral benzodiazepine (PBZ) receptors have recently been shown to be abundant in brain tumors. It was also reported that antagonist which binds to the PBZ receptors can reduce the rate at which cells grow in the tissue culture from brain tumors proliferate.

Here we report measurements of the B_{max} and K_D on brain tumor surgical specimens. The measurements were done on six malignant gliomas (grade III and IV) and two low grade gliomas (grade II). The surgical specimens were cut into 15 μm thick slices, mounted on gelatine coated microscope glasses and incubated for 60 min in baths containing increasing concentrations of PK-11195 and constant amounts of [^{3}H]-labelled PK-11195. Non-specific binding was done in the bath with 1 μM of PK-11195. The kinetic experiments, both association and dissociation, were done at 1 nM. K_D estimates obtained from the kinetic and saturation measurements agreed well. In malignant brain gliomas we found only one receptor site, however, in the grade II astrocytomas both kinetic and saturation measurements showed the presence of two receptor sites. In malignant gliomas K_D and B_{max} were 7.1 $\pm$ 0.1 nM and 1.15 $\pm$ 0.16 nmol/g-brain, respectively. Two sites in grade II astrocytomas showed a K_D of 1.8 and 23.7 nM, with corresponding B_{max} of 0.1 and 2.1 nmol/g-brain. For the normal human cortex we found a K_D of 15 $\pm$ 2 nM and a B_{max} of 0.4 $\pm$ 0.1 nmol/g-brain. This work was supported by the USPHS grant NS-22230.

470.10

CHARACTERIZATION OF PAF-INDUCED ELEVATION OF CYTOSOLIC FREE CALCIUM LEVEL IN NEUROHYBRID NCB-20 CELLS. T.L.Yue*, M. Gleason* and G. Feuerstein, Dept. of Pharmacology, SmithKline Beecham, King of Prussia, PA 19406-0939

The effects of platelet-activating factor (PAF) on intracellular level of free Ca^{2+} ($[Ca^{2+}]_i$) were studied in neurohybrid NCB-20 cells. In fura-2-loaded NCB-20 cells, PAF induced immediate and concentration-dependent increase in $[Ca^{2+}]_i$ a maximum increase of 334$\pm$27nM Ca^{2+} (n=25). PAF-induced $[Ca^{2+}]_i$ mobilization was inhibited by PAF antagonist, BN50739, WEB2086, SRI63-441 and BN52021, with IC_{50} of 12, 38, 897 and 45000nM, respectively. Calcium channel blockers had no effect on PAF-induced increase in $[Ca^{2+}]_i$. Extracellular Ca^{2+}-depletion reduced a large fraction (70%) of PAF-induced increase in $[Ca^{2+}]_i$, suggesting the majority of $[Ca^{2+}]$ mobilization was originated from extracellular milieu; however, a small portion (30%) was originated from intracellular sources, which was inhibited by TMB-8. NCB-20 cells exhibited a homologous desensitization to sequential addition of PAF, but no heterologous desensitization between PAF and bradykinin or ATP was observed. These data suggested that PAF-induced neuronal receptor activation results in increased $[Ca^{2+}]_i$ via a non voltage calcium channel mechanism and intracellular Ca^{2+} release.

PEPTIDES—RECEPTORS, METABOLISM AND ACTIONS

471.1

EFFECTS OF ANGIOTENSIN CONVERTING ENZYME INHIBITORS (CEI) ON BRAIN ANGIOTENSIN II (AII) BINDING. K.H. Berecek and B.H. Swords* Hypertension Res., Univ of Alabama, Birmingham, AL 35294

Brains and neuronal cells from SHR have an increased number of AII receptors in comparison to controls. In order to determine whether there was an alteration in AII binding in SHR after CEI, we compared ^{125}I AII binding in neuron-enriched primary cultures of whole brains from 1 day old SHR pups treated in utero with Captopril (CAP) vs control SHR (CON) pups and studied the effect of short term incubation of neuronal cells from CON and CAP SHR and normotensive rats (NR) with CAP or lisinopril (LIS). ^{125}I AII binding in neuronal cultures from CAP SHR was decreased when compared to cells from CON SHR. Scatchard analysis revealed no differences in Kd but Bmax was less in CAP SHR (7.5) than CON SHR (9.3 fmol/mg protein). Short term (24 h) incubation of cells with CAP or LIS (10^{-5}M) did not affect AII binding in neuronal cultures from NR. AII binding was decreased in SHR cells treated with CAP (CON SHR: Kd = 0.28 nM, Bmax 9.3 fmol/mg protein vs CON SHR + CAP: 0.3, 7.5) moreover, a significant decrease occurred by 1/2 h of incubation. AII binding was also decreased in SHR treated in utero with CAP (CAP SHR 0.32, 7.1; CAP SHR + CAP 0.29, 6.1) but to a lesser extent than CON SHR. These data suggest that CEI decrease ^{125}I AII binding in brain from SHR by competitive inhibition or receptor internalization and the AII receptor in SHR may be regulated differently from that of NR.

471.2

AUTORADIOGRAPHIC LOCALIZATION OF ANGIOTENSIN II RECEPTOR SUBTYPES IN THE RAT BRAIN. B.P. Rowe, K.L. Grove*, D.L. Saylor* and R.C. Speth. Dept. Physiology, Col. of Med., East Tennessee State Univ., Johnson City, TN 37614 and Dept. VCAPP, Washington State Univ., Pullman, WA 99164-6520.

Recent studies with new, selective angiotensin II (AII) antagonists have revealed AII receptor subtypes (Chiu et al, Biochem. Biophys. Res. Comm. 165: 196, 1989). Since these receptors can be discriminated by their binding sensitivity to sulfhydryl reducing agents, and our studies indicate that central AII receptors are influenced differentially by mercaptoethanol (M) in the rat (Rowe et al, FASEB J. 4: A600, 1990), we reasoned that central AII receptors occur as different subtypes also. We conducted competitive autoradiography experiments with the non-peptidic AII receptor subtype selective antagonist, DuP753 (gift from duPont). Rat brain sections were incubated with 240-270pM ^{125}I sar^1 ile^8 AII (^{125}I SIAII) alone, with AII (10^{-6}M), or with DuP753 (10^{-4} - 10^{-8} M). ^{125}I SIAII binding was displaced by 10^{-6}M DuP753 (designated AII_α receptor subtype) at some sites, but was unaffected by 10^{-4} M DuP753 at other sites (AII_β subtype). Based upon the selective competition with DuP753 we have categorized nuclei as having predominantly AII_α or AII_β subtypes as follows: AII_α: solitary tract nucleus (n), parabrachial n., anterior pituitary, para and periventricular hypothalamus, subfornical organ, organum vasculosum, and median eminence. AII_β: inferior olive, locus coeruleus, inferior and superior colliculus, most thalamic areas, and medial amygdala. ^{125}I SIAII binding is inhibited by M at AII_α sites but not AII_β sites. [Supported by Amer. Heart Assoc., Tenn. Affiliate and NIH (NS-24388)].

471.3

ANGIOTENSIN III AND P-AMINOPHENYLALANINE^6 ANGIOTENSIN II DISPLAY SELECTIVITY FOR BRAIN AII_β RECEPTOR SUBTYPE BINDING SITES. K.L. Grove, B.P. Rowe and R.C. Speth, Dept. of VCAPP, Washington State University, Pullman, WA 99164-6520 and Dept. Physiol., E. Tenn. State Univ. Johnson City, TN 37614

Competition binding studies with selective antagonists and differential effects of sulfhydryl reducing agents indicate subtypes of angiotensin II (AII) receptors: a DuP 753-sensitive site (AII_α) and a DuP 753-insensitive site (AII_β, see Rowe et al., this volume). Competition for the binding of ^{125}I-sarcosine^1, isoleucine^8 AII (^{125}I-SI AII, 300 pM) to 20 μ-thick rat brain sections by the selective AII_α antagonist (DuP 753, 5 x 10^{-7} M) was compared to that for the heptapeptide, angiotensin III (AIII, 100 nM) and p-aminophenylalanine^6 AII (pNH_2F AII, 1 μM) using quantitative densitometric analysis of autoradiograms. Both AIII and pNH_2F AII displaced ^{125}I-SI AII binding in brain regions where AII_β receptors predominate, e.g., septum, medial amygdala, thalamus, subthalamus, colliculi, locus coeruleus and inferior olive, but it was a poor competitor for ^{125}I-SI AII binding in brain regions where AII_α receptors predominate, e.g., circumventricular organs, median preoptic nucleus, hypothalamus, piriform cortex, solitary tract nucleus, vagal motor nucleus, spinal trigeminal tract. This indicates that agonists, including the endogenous peptide AIII, can distinguish brain region-specific AII receptor subtypes. Supported by NIH (NS24388) and Am. Heart Assn., TN affiliate. DuP 753 was a gift from Dr. Pieter Timmermans (Dupont).

471.4

COUPLING BETWEEN MITOCHONDRIAL BENZODIAZEPINE RECEPTORS AND HORMONE STIMULATED STEROIDOGENESIS. K.E. Krueger, A.G. Mukhin*, and V. Papadopoulos* Fidia-Georgetown Institute for the Neurosciences and Dept. of Anatomy & Cell Biology, Georgetown Univ. Med. Sch., Washington, D.C. 20007.

Our previous studies demonstrated that peripheral-type benzodiazepine receptors (PBR) regulate steroid synthesis by mediating cholesterol transport within mitochondria. In these studies we have used the ACTH-responsive Y-1 adrenocortical and the hCG-responsive MA-10 Leydig cell lines to examine the relationship between PBR and hormone-stimulated steroidogenesis. Most PBR ligands tested had no effect on steroidogenesis by ACTH or hCG, however, flunitrazepam, at submicromolar concentrations, inhibited steroid production induced by these hormones in Y-1 and MA-10 cells, respectively, or by 1 mM dibutyryl cyclic AMP. This inhibition by flunitrazepam was characterized by a decrease in the efficacies but not in the potencies of the hormones suggesting that this benzodiazepine affected a step following activation of the hormone receptors. Higher affinity PBR ligands such as PK 11195 and Ro5-4864, at concentrations which were 10-50 fold lower than those of flunitrazepam, blocked this inhibition. These findings support the possibility that hormone-stimulated steroidogenesis includes a mechanism involving the direct participation of PBR, for which flunitrazepam, a low intrinsic activity agonist, may act as an antagonist.

471.5

TWO PROLACTIN CELL SUBPOPULATIONS IN THE LACTATING RAT EXPRESS DIFFERENT SUBTYPES OF D2 RECEPTORS. L.A. Kukstas*, V. Hanin*[1], J. Demaille[1]*, C. Domec[2]*, J. Bonner[2]*, J.M. Israel*, and J.D. Vincent, INSERM U176, rue St. Saëns, Bordeaux 33077. [1] CRBM, CNRS, rte de Mende, Montpellier 34033. [2] IBCN, CNRS, rue St. Saëns, Bordeaux.

Dopamine (DA) the major prolactin (PRL) inhibiting factor has been shown to act by different mechanisms in the two subpopulations of PRL cells present in the adenohypophysis of the lactating rat (Israel *et al.* Neuroendocrinology 51 p113 1990). Since two subtypes of D2 DA receptors have been recently described (Monsma *et al* Nature 342, Dec 1988), their implication in this discrepancy has been investigated. Bidimensional electrophoresis of total cell phosphorylated proteins followed by autoradiography confirmed the presence of the D2 receptor in both subpopulations of PRL cells as expected from electrophysiological studies. An antibody was produced against a sequence of 18 amino acids within the D2 receptor sequence (Bunzow *et al* Nature 336 Dec 1989). This antibody was shown to recognise one of the two D2 subtypes. Western blots of proteins extracted from the two PRL cell subpopulations were analysed with this antibody, and the receptor was revealed for only one of them, suggesting that each of the two PRL cell subpopulations expresses a different D2 receptor subtype. Finally, the polymerase chain reaction (PCR) was applied to cDNA prepared from the two PRL cell subpopulations; the primers for the PCR were synthetic oligonucleotides (30 mer) complementary to sequences widely flanking the differentially expressed part of the D2 receptor. Electrophoresis of the reaction media after PCR showed the length of the amplified sequences from the two cell types to differ by approximately 100 nucleotides. This corresponds to the difference expected for the two D2 subtypes. These results strongly suggest that two subpopulations of lactotroph express two types of D2 receptor. As it is hypothesised that the two subtypes bind with different G proteins, these results could account for the distinct mechanisms brought into play by DA in the two subpopulations.

471.6

MELATONIN RECEPTORS ARE PRESENT IN THE FERRET PARS TUBERALIS AND PARS DISTALIS, BUT NOT IN BRAIN. D. R. Weaver and S. M. Reppert, Laboratory of Developmental Chronobiology, Children's Service, Massachusetts General Hospital & Harvard Medical School, Boston MA 02114.

The distribution of melatonin receptors varies widely among mammalian species. We continue to examine melatonin receptor distribution in photoperiodic species in an effort to identify conserved sites of melatonin action.

Three female ferret brains with pituitary attached were generously provided by Dr. K.D. Ryan, U Pittsburgh Med Sch. Coronal sections (20 micron) at 120 micron intervals throughout the brain were processed for autoradiographic localization of 2-125I-iodomelatonin (I-MEL) binding as previously described (J Neurosci 9: 2581-2590, 1989).

Specific binding of I-MEL (40 pM) was observed only in the pars tuberalis (PT) and pars distalis (PD) of the pituitary. The entire PT was intensely labeled. Specific binding also extended throughout the PD, at levels lower than in the PT. There was no specific labeling in brain.

The consistent presence of high-affinity melatonin receptors in the PT of all photoperiodic species examined so far suggests that the PT plays a major role in mediating the effects of melatonin on the hypothalamic-pituitary-gonadal axis. The absence of melatonin receptors in ferret brain reinforces the hypothesis that neural sites of melatonin binding may not be necessary sites of melatonin action for the regulation of reproduction.

471.7

DIFFERENTIAL EXPRESSION PATTERNS OF A MOLLUSCAN INSULIN-RELATED GENE FAMILY IN NEUROENDOCRINE CELLS OF *LYMNAEA STAGNALIS*. W.P.M. Geraerts[1], A.B. Smit[1], K.W. Li[1], A. ter Maat[1] and H. van Heerikhuizen[2]. Depts. of Endocrinology[1] and Biochemistry[2], Free University, De Boelelaan 1087, 1081 HV Amsterdam, The Netherlands

A gene family that codes for unique and remarkably diverse precursors of molluscan insulin-related peptides (MIPs) has been identified in the snail, *Lymnaea stagnalis*. All members of the MIP gene family have an intron/exon organization similar to the one found in vertebrate insulin genes, with 3 exons and 2 introns. Exon 2 encodes the signal peptide, the B chain and a few amino acids of the C-peptide(s), whereas exon 3 encodes the remainder of the C-peptide(s) and the A chain. The sequence diversity among MIPs is much larger than that observed among vertebrate insulins. A model is presented which explains the extraordinary MIP diversity by the occurrence of macroscale evolutionary events involving exchanges of parts of the MIP genes by intergenic crossing-over. Four MIP genes are expressed in the 200 cerebral neuroendocrine light green cells (LGCs), which control growth and associated metabolic processes. We show that various environmental conditions result in a strongly altered steady state concentration of the different MIP-RNAs, suggesting that the MIP genes are subjected to a stimulus-specific transcriptional regulation. Pulse-chase experiments show that the C-peptides are proteolytically cleaved out of the precursors, thus giving rise to mature 2-chain MIPs. Physiological concentrations of D(+) glucose induce spiking activity in the LGCs and a concomitant release of MIPs. This creates an interesting parallel with the β cells of the pancreatic islets in vertebrates

471.8

APOMORPHINE STIMULATES OXYTOCIN RELEASE IN MALE AND FEMALE MONKEYS. I.A. Amico, S.M. Pomerantz, L.M. Layden* and I.L. Cameron, Depts. of Medicine, Physiology and Psychiatry, Univ. of Pittsburgh School of Medicine and Oakland VAMC, Pittsburgh, PA 15260.

In male rats the dopamine receptor agonist, apomorphine, has been shown to stimulate the release of oxytocin from the neurohypophysis and this release is associated with increased yawning and penile erection. The present study was designed to investigate whether dopaminergic agents may exert similar neuroendocrine and behavioral effects in primate species. Three female rhesus monkeys and one male cynomolgus monkey were implanted with chronic indwelling venous catheters and outfitted with standard jacket/tether/swivel systems to allow remote blood sample collection. Blood samples were collected at 15 and 1 min prior to administration of an i.v. injection of apomorphine and at intervals 1 to 120 min after apomorphine. Low doses of apomorphine (25-100 μg/kg) which reliably elicited yawning generally failed to alter plasma oxytocin secretion over the two hour post-apomorphine sampling period. By contrast, higher doses of apomorphine (200-400 μg/kg) elicited stereotypic behaviors (e.g. gnawing, scratching, head-bobbing) in the monkeys as well as a dose-dependent stimulation of oxytocin secretion. Apomorphine's stimulation of both oxytocin secretion and stereotypy was extremely rapid with an initial increase being observed within two minutes of apomorphine administration and a peak response generally occurring within 10-15 min following apomorphine administration. These results indicate that apomorphine acts as a potent stimulus to release oxytocin from the neurohypophysis in male and female monkeys and is therefore likely to be a useful pharmacological tool for further studies exploring the physiological and behavioral actions of oxytocin in primate species.

471.9

SURVIVAL OF VASOPRESSIN (AVP) AND OXYTOCIN (OT) NEURONS IS IMPAIRED BY HYPONATREMIA IN RATS WITH PITUITARY STALK INJURY. Janos Dohanics, G. E. Hoffman, and J. G. Verbalis Deparments of Medicine & Physiology, University of Pittsburgh, Pittsburgh, PA 15261.

We have recently reported that compression of the pituitary stalk (SC) results in a functional neurolobectomy; 21 days after this lesion approximately 70% of AVP and 30% of OT neurons died in the supraoptic (SON) and paraventricular nuclei (PVN). Other studies have shown that loss of AVP but not OT neurons following neurolobectomy is greater in rats treated with DDAVP. However, since DDAVP by itself does not change metabolic activity in magnocellular neurons, in this study we determined the effect of chronically altered metabolism on the survival of magnocellular neurons following SC. Chronic hyponatremia (CH) was chosen, because such rats are known to have markedly inhibited AVP and OT secretion in response to osmotic and volemic stimulation, as well as decreased synthesis of AVP and OT mRNA. CH was induced in adult rats fed a nutritionally balanced liquid diet as their only food source and infused with DDAVP (5 ng/h s.c.). After 10 days of sustained hyponatremia SC was performed, and 6 days later plasma [Na^+] was slowly increased to the normonatremic range. Following correction of CH, water intake of SC rats exceeded 500 ml a day. Twenty-one days after SC rats were perfused with Zamboni fixative and the brains stained for AVP-neurophysin and OT-neurophysin immunoreactivities. The table below shows the percentage of surviving neurons:

Treatment	AVP-NP		OT-NP	
	SON	PVN	SON	PVN
Sham operated	100 %	100 %	100 %	100 %
SC	33 %	35 %	70 %	58 %
Hyponatremic+SC	1.3 %	6.5 %	13.6 %	26.6 %

Thus, while DDAVP treatment alone decreases survival rates of only AVP neurons following axonal injury, CH markedly impairs survival of both AVP and OT neurons. These results therefore demonstrate that suppressed metabolic activity represents an additional risk factor for neuronal survival following injury.

471.10

CATECHOLAMINE DEPLETIONS OF THE DIAGONAL BAND OF BROCA (DBB) ATTENUATE BARORECEPTOR SENSITIVITY OF RAT SUPRAOPTIC (SON) VASOPRESSIN NEURONS. J.T. Cunningham, R. Nissen, & L.P. Renaud, Neuroscience Unit, Ottawa Civic Hospital, Ottawa, Canada K1Y 4E9.

In the rat, stimulation of peripheral baroreceptors by acute drug-induced increases in blood pressure will transiently inhibit the spontaneous activity of SON vasopressin-secreting neurons. Previous research indicates this response is mediated via the DBB. The present study evaluated the importance of the catecholaminergic innervation of the DBB in the barorecepetor senstivity of vasopressin neurons in the SON. Adult male Long Evans rats anesthetized with nembutal were injected in the DBB with 6-OHDA (4 ug/2 ul). Controls were injected with either vehicle (0.1% ascorbic acid in 0.9% NaCl) or the same dose of 6-OHDA after administration of the norepinephrine uptake inhibitor desmethylimpramine (DMI, 25 mg/kg ip). Two weeks later, the rats were reanesthetized with nembutal and prepared for extracellular recording of identified neurons in the SON using a transpharyngeal approach. In vehicle and DMI control experiments, all phasically-firing vasopressin neurons were inhibited by increases in blood pressure produced by metaraminol (10 ug/10 ul iv). Equivalent metaraminol-induced pressor responses inhibited less than half of the phasic vasopressin neurons (8/18) in rats with 6-OHDA injected into the DBB. This decrease in baroreceptor sensitivity in the rats injected with 6-OHDA was significantly different from vehicle injected controls ($X^2(df=1)=4.07$, $p < 0.05$) and from DMI pretreated rats ($X^2(df=1)=8.64$ $p < 0.05$). The results suggest that the noradrenergic innervation of the DBB is involved in the baroreceptor inhibition of vasopressin neurosecretory neurons in the SON. (Supported by NRSA MH0977, FRSQ and the MRC of Canada)

471.11

STRONG INTRINSIC INHIBITION IN CAT MAGNOCELLULAR NEUROENDOCRINE CELLS. R.D. Andrew and M. Fagan. Dept. of Anatomy, Queen's University, Kingston, Ontario. K7L 3N6.

Most magnocellular neuroendocrine cells (MNCs) in the cat supraoptic nucleus (SON) synthesize and release vasopressin systemically from their neurohypophysial axon terminals. Unlike in rat, cat MNCs discharge slower and are incapable of the phasic firing that facilitates vasopressin release. The reasons were examined using intracellular recording from feline and rat SON in coronal hypothalamic slices.

Cat MNCs displayed a higher mean input resistance (301 MΩ,n=56) than rat (150 MΩ,n=105). Spike hyperpolarizing afterpotentials were 16.5±0.5 mV mean amplitude, more than twice that of rat. An evoked spike train was followed by a prominent afterhyperpolarization (AHP) several times the duration of its counterpart in rat. The AHP reversed near the equilibrium potential for K^+, represented an increased membrane conductance and was eliminated in low Ca^{2+} saline or by the $I_{K(Ca)}$ blockers apamin (100 nM) or d-tubocurarine (200 μM). Both blockers decreased spike frequency adaption but did not unmask phasic firing, confirming that cat MNCs lack a regenerative burst mechanism. Increasing osmolality by 10-85 mOsm increased firing in 75 of 85 rat units and in 120 of 150 cat units. Phasic firing evolved in 47% of slow units in rat but no cat units became phasic. We conclude that a prominent $I_{K(Ca)}$ helps cat MNCs resist bursting and rapid firing. However their osmosensitivity is comparable to rat and therefore independent of the current that drives phasic firing in rat. This study illustrates that dramatic differences in the intrinsic properties of homologous neurons can exist across mammalian species.

471.12

A MINIMAL MODEL OF NEUROHYPOPHYSEAL HOMEOSTASIS. M.D. Fitzsimmons, M.M. Roberts, A.G. Robinson. Division of Endocrinology. University of Pittsburgh. PA. 15261.

Posterior pituitary vasopressin (AVP) content is dynamic and depends on physiologic conditions; prolonged stimulation can reduce store size to 5-10% of control levels, while removing the stimulus allows recovery to 100% basal or greater. Over a broad range of stimulus conditions, the rate of AVP synthesis in rats remains directly proportional to AVP mRNA levels. These results suggest that magnocellular neurons may regulate vasopressin synthesis exclusively through transcriptional mechanisms. We tested whether regulation at a single site (transcription) explains broad shifts in pituitary content using a computer model of vasopressin synthesis. Mathematical analysis reveals two central characteristics of the model: 1) solely transcriptional regulation can only achieve equilibrium if the rate of mRNA transcription varies linearly with hormone release rate and 2) the dynamics of neurohypophyseal hormone storage depend heavily on AVP mRNA half-life (the longer the half-life, the slower the synthetic response). Our half-life estimate (4.6 days) comes from experimental measurements.

Although computer simulations of this minimal model coincided well with pituitary depletion resulting from increased release, the model underestimated the rate of recovery of stored hormone after prolonged stimulation. Thus, we tested several modifications to the model: decreased hormone release during recovery, non-linear transcription, and mRNA half-life regulation. Cytoplasmic control of AVP mRNA decay proved the most promising modification for describing the time course of pituitary recovery. By mimicking physiological conditions, the computer model of transcriptional regulation provides a theoretical basis for observed dynamics of AVP mRNA and pituitary AVP content and allows development of testable hypotheses.

PAIN: PATHWAYS II

472.1

ONGOING INPUT FROM A PUTATIVE FOCUS OF NOCICEPTOR DISCHARGE MAINTAINS THE ABNORMAL PAIN SENSATIONS OF REFLEX SYMPATHETIC DYSTROPHY. S. Lynch, R.H. Gracely*, and G.J. Bennett. Neurobiology and Anesthesiology Branch, NIDR, NIH, Bethesda, MD 20892.

Many patients with reflex sympathetic dystrophy (RSD) perceive light tactile stimuli to be very painful. It is known that in many cases this mechanical allodynia is dependent upon input from Aß low-threshold mechanoreceptors (AßLTM). We have found that AßLTM-dependent mechanical allodynia can be eliminated transiently (thereby normalizing touch-evoked sensations) by lidocaine infusion into a putative focus of nociceptor discharge in the affected extremity. Our observations are illustrated by an advantageous case with a painful focus that was distant from an area of allodynia. The patient had spontaneous pain and mechanical allodynia in the left forearm and hand. The pain was of 23 months duration and appeared subsequent to an ulnar nerve transposition at the elbow. The allodynia was shown to be AßLTM-dependent by testing with v. Frey hairs and transcutaneous electrical stimulation before and after a differential ischemic block. Palpation of the painful focus (an atrophic, hyperpigmented area just distal to the surgical scar) caused intense shooting pains throughout the arm and hand. Completely anesthetizing this region with subcutaneous 1.5% lidocaine relieved the spontaneous pain and mechanical allodynia in all areas. Testing detection thresholds for warm, cold, hot, v. Frey hair, and electrical stimuli showed that the relief was not due to the spread of lidocaine to the innervation of the areas that previously exhibited allodynia. Allodynia and spontaneous pain returned along with the return of sensibility to the injected area. On a separate occasion, lidocaine given in the other arm had no effect on the painful side, showing that the pain relief was not due to a systemic drug effect.

Such observations support a model that proposes that an ongoing nociceptor input maintains a central processor in an abnormal state. The abnormal central state is dynamic - when the nociceptor input is blocked, it reverts to normal. The ongoing nociceptor input may be driven, completely or in part, by sympathetic activity and it may arise from several sources (e.g., neuroma discharge, poorly healed joint injury).

472.2

SIGNALLING OF A STEP-LIKE CHANGE IN INTENSITY OF A NOXIOUS MECHANICAL STIMULUS: SEPARATING "SENSORY" FROM "AUTONOMIC" RAT SPINAL DORSAL HORN NEURONES Jennifer M.A. Laird & Fernando Cervero* Dept.of Physiology, University of Bristol Medical School, Bristol, BS8 1TD, U.K.

A noxious stimulus evokes a variety of responses; sensory (pain), motor (withdrawal reflex) and autonomic (cardiovascular, etc). Peripheral nociceptors provide the input to all of these systems. The first possible point of division is the dorsal horn of the spinal cord. However, which dorsal horn cells participate in the different systems is not clear. An approach to this problem was suggested by the results of experiments in which human subjects rated the intensity of the pain sensation produced by a noxious mechanical pinch applied to an interdigital web. The 120s stimulus had a step-like increase or decrease in intensity after 60s. Subjects detected a step increase but rarely a step decrease. Skin vasoconstrictor activity was also measured and found to follow both the step increase and the step decrease.

We have now investigated the ability of neurones in the sacral dorsal horn of the rat to signal step changes in 120s pinch stimuli. Four pinches, 2 with a step increase and 2 with a step decrease in intensity (4N-6N, 6N-8N, 8N-6N and 6N-4N), were delivered in random order to the neurone's receptive field on the tail. Cells with a nociceptive input, either exclusively (class 3) or in addition to a low threshold input (class 2) fired throughout the 120s pinch. The 26 cells could be divided into 4 groups depending on their responses to the pinch stimuli as follows: i) signalling both a step up and a step down (n=13) ii)signalling only a step up (n=7) iii) signalling only a step down (n=4) iv) signalling neither (n=2). Each of these groups contained both class 2 and class 3 cells, located throughout the dorsal horn.

These results show that there are groups of dorsal horn neurones that follow the autonomic and the sensory output patterns. We conclude that different sub-groups of class 2 and 3 neurones appear to be involved in both sensory and in autonomic aspects of nociception.

472.3

LAMINA I TRIGEMINOTHALAMIC PROJECTIONS IN THE MONKEY. A.D. Craig, Div. of Neurobiology, Barrow Neurological Institute, Phoenix, AZ 85013

Thermoreceptive and nociceptive lamina I neurons constitute half of the spinal input to the thalamus, and their termination sites signify important loci for temperature and pain sensation. Lamina I projections from the trigeminal dorsal horn have been identified in the cynomolgus monkey by using the PHAL anterograde tracing method previously employed successfully in the cat. Dense terminal clusters bearing large boutons are concentrated at the ventrocaudal (probable nociceptive region) and dorsomedial (possible thermoreceptive region) aspects of VPM. Strong medial thalamic terminations in the ventral aspect of caudal MD (adjacent to Pf), which could be homologous to the submedial projection in the cat, are similar to the "paralaminar MD" projection described by Ganchrow (JCN 178:281, 1978) in the squirrel monkey. These findings offer unambiguous, substantive evidence for the location of clinically critical thalamic sites for trigeminal pain sensation.

Supported by NS25616 and the Barrow Neurological Foundation.

472.4

RESPONSES OF SPINOTHALAMIC AND SPINORETICULAR NEURONS TO INTRACARDIAC INJECTION OF PHENYLBIGUANIDE, A 5-HT$_3$ RECEPTOR AGONIST. R.W. Blair, D.C. Bolser, and R.D. Foreman. Univ. Okla. Hlth. Sci. Ctr., Okla. City, OK 73190, and Shering-Plough Corp., Bloomfield, NJ 07003.

5-HT$_3$ receptors are located on vagal afferents; activation of these receptors produces a Bezold-Jarisch-like response. The purpose of this study was to determine whether phenylbiguanide (PBG), a 5HT$_3$ agonist, could influence spinal neurons by activating cardiac sympathetic afferents. Nine cats and 10 monkeys were anesthetized with chloralose and/or pentobarbital. Electrophysiological techniques were used to record activity of thoracic spinothalamic (N=17, STT), spinoreticular (N=6, SRT), STT-SRT (N=1), or non-projecting (N=4) neurons. PBG was injected into the left atrium using doses that had little or no effect on neuronal activity when injected into the ascending aorta; the most effective doses were 30-40 μg/kg. In response to PBG, 17 neurons were excited, 6 were inhibited, and 5 were unaffected. Three responsive neurons were tested pre- and post-vagotomy. Two of these cells were excited by PBG, and the response was comparable or bigger after vagotomy. The other cell was inhibited, and the inhibition blocked by vagotomy. In 10 neurons responsive to PBG and assessed for responses to left vagal afferent stimulation, the vagal response was in a different direction than the PBG response. Taken together, the results suggest that thoracic STT and SRT cells are influenced by 5-HT$_3$ mediated excitation of cardiac sympathetic afferents. (Supported by NIH grants HL22732 and HL29618)

472.5

SPINALL NEURONS WITH BRANCHED AXONS PROJECTING TO THE SOLITARY TRACT AND DORSAL COLUMN NUCLEI. G.W.Lu, Z.Meng*, L.Luo* and Y.Yamakami*. Dept. of Neurobiol. Capital Institute of Medicine, Beijing. 100054, China.

Retrograde labeling and antidromic activation techniques were used to identify the dual projection of spinal neurons to both the solitary tract nucleus(STN) and dorsal column nuclei(DCN).

Experiments were conducted on adult rats anesthetized with sodium-pentobarbital. Propidium iodide(PI) and Bisbenzimide (Bb) were injected into the STN and DCN respectively in 10 rats. Antidromic stimulation was alternatively delivered to STN and DCN in another group of 26 rats. The distribution of PI and Bb labeled cells and STN and DCN activated neurons were respectively identified in the lumbosacral dorsal horn.

Two hundred and eighty two cells were found to be labeled by PI and/or Bb in the laminae III-VI. Eighteen percent of the sample (51 cells) were found to be labeled by both PI and Bb. A total of 92 neurons in laminae III-VI were found to be driven by stimulation of the STN and/or DCN. Of them, 38 and 54 neurons were found to antidromically and synaptically respond to both the STN and DCN stimulation. Synaptic responses were also shown in 8 of the 38 neurons that responded antidromically to both the STN and DCN.

These results indicate that some spinal neurons issue branched axons projecting to both the STN and DCN and some of these dual projection neurons are innervated in turn by the STN and/or DCN. In addition, some spinal neurons are doubly innervated from both the STN and DCN.

472.6

PHYSIOLOGY AND MORPHOLOGY OF NOCIRECEPTIVE AND THERMORECEPTIVE PARABRACHIAL NEURONS. RM Slugg and AR Light, Dept. Physiology, UNC-Chapel Hill, Chapel Hill, NC 27599.

Neurons in the parabrachial nucleus were examined for cutaneous input in anesthetized rats. Of the 90 neurons characterized, 71 (79%) responded to noxious stimuli. Of these 71 neurons, 50 were excited only by noxious mechanical or noxious mechanical and noxious thermal stimuli, and 5 were excited only by noxious thermal stimuli. Receptive field (RF) size ranged from a small area on one limb to the entire body surface. Most RF's were contralateral or, if bilateral, had a greater responsiveness from contralateral stimuli. Of the remaining neurons, 17 (19%) were classified as innocuous cooling, and 2 (2%) as slow brush. Fifteen neurons were intracellularly labeled with HRP, biocytin, or neurobiotin. All labeled neurons were within the lateral parabrachial nucleus, and did not appear to be segregated by RF or modality. These neurons were characterized by a few long thin dendrites, which extended beyond the border of the subnucleus in which the soma was located, either rostrocaudally, dorsoventrally, or mediolaterally. Labeled axons were extremely thin, and projected medially and rostrally but were not observed to cross midline. Thus, neurons in the lateral parabrachial nucleus may act as specific relays for nociceptive and thermoreceptive information. Supported by grants PHS DA04420 and NS16433.

472.7

PROJECTIONS TO THE VENTRAL PERIPHERY OF THE CAT'S THALAMIC VENTRAL POSTEROMEDIAL NUCLEUS (VPM$_{vp}$). C. Vahle-Hinz and K.-D. Kniffki. Physiologisches Institut, Universität Würzburg, D-8700 Würzburg, FRG.

The VPM$_{vp}$ is a thalamic nucleus in the nociceptive system of the cat. Neuronal tracers (HRP, WGA-HRP, WGA-HRP-gold) were injected into this region to retrogradely label the neurons of origin of its inputs from brainstem, thalamus and cortex.

All tracers used produced similar results, however, the number of labeled neurons was substantially higher with WGA-HRP-gold. Retrogradely labeled neurons were found contralateral to the injection site in the spinal trigeminal complex, especially in the marginal laminae of nucleus caudalis, in the ventral principal trigeminal nucleus and in the periaqueductal grey. Ipsilateral to the injection neurons were labeled in the dorsal principal trigeminal nucleus, the parabrachial complex, the periaqueductal grey, the thalamic reticular nucleus and the cingulate cortex.

Since the VPM$_{vp}$ is small and several pathways traverse this region, some spread of the tracer to VPM proper and retrograde labeling via damaged fibers of passage have to be taken into account. However, the responsiveness of VPM$_{vp}$ neurons to noxious stimuli on the head, to stimulation of visceral organs and baro- and chemoreceptors suggests that fibers from the spinal trigeminal and parabrachial nuclei may serve these inputs and terminate within VPM$_{vp}$. In addition, neurons from the cingulate cortex, the thalamic reticular nucleus and the periaqueductal grey may exert modulatory influences.

Supported by the Deutsche Forschungsgemeinschaft.

472.8

THALAMIC PROCESSING OF CRANIOVASCULAR PAIN IN THE CAT: A 2-DEOXYGLUCOSE STUDY. P.J. Goadsby and A.S. Zagami[1]. The National Hospitals for Nervous Diseases, Maida Vale, London England & [1]Institute of Neurological Sciences, The Prince Henry Hospital, Sydney, Australia.

The superior sagittal sinus (SSS), as well as other cranial vessels and the dura mater, is known to be pain sensitive in man. Studies of the central processing of pain from intracranial structures have focused on the first order neurons of the trigeminal system that synapse in the trigeminal nucleus. We have shown that electrical stimulation of the SSS causes metabolic activation of the trigeminal system and have now extended these data by assessing the thalamus. The SSS was electrically stimulated in the chloralose-anesthetized (60mg/kg,ip), paralysed and ventilated cat. Metabolic activity in the thalamus was determined using the 2-deoxyglucose method with quantitative tissue autoradiography. In a group of animals metabolic activity was measured after bilateral trigeminal ganglia abalation. Stimulation of the SSS in the intact cats increased glucose utilization in the ventrobasal complex of the thalamus by 160% while no change was seen in surrounding structures including the lateral geniculate nucleus or cerebral cortex. This response was blocked by bilateral abalation of the trigeminal ganglia. These data are the first demonstration of metabolic activation in second order neurons in the trigeminovascular system. Furthermore these data clearly implicate the ventrobasal complex in processing craniovascular nociception.

472.9

NOCICEPTION AND NOCICEPTIVE MODULATION OF TASK-RELATED RESPONSES IN AREA 7B CORTEX OF MONKEYS. W.K. Dong and V.J. Roberts. Department of Anesthesiology and Multidisciplinary Pain Center, University of Washington School of Medicine, Seattle, WA 98195.

Three distinct functional groups of neurons with somesthetic responses have been identified in the inferior parietal lobule (area 7b) of monkeys that performed an appetitive tolerance-escape task. Monkeys were allowed to initiate and terminate thermal stimulation of the face and were reinforced for completing trials that sometimes required tolerance of noxious temperatures. A nociceptive group of neurons had peak discharge frequencies that increased monotonically in response to graded thermal pulses. Maximal discharge frequencies were at temperatures near the pain tolerance level (~50% escapes). A task-related group of neurons displayed inhibition of background activity during button pressing which led to reinforcement. This may be a nonspecific mechanism for decreasing background noise and enhancing neural responses to an anticipated perceptual event (i.e., cued button release or cued food reinforcement). A reversal of this inhibitory period by presentation of tolerable warming pulses was seen in some task-related neurons, but optimal reversal of inhibition required intolerable warming pulses at the pain tolerance level in other task-related neurons. A third group of neurons had both trigeminal somatosensory and visuosensory properties; their cutaneous and visual receptive fields were aligned along common spatial coordinates. Slowly adapting discharges were elicited by a visual stimulus approaching and staying near a face cutaneous receptive field. These groups may be part of a polysensory neuronal assembly that processes information used to guide the body away from stimuli that threaten tissue injury or to strengthen learned strategies that avoid or tolerate noxious stimuli. Supported by NIH grants DE05130, DE07617 and NS07217.

SEROTONIN IV

473.1

LONG-TERM ADMINISTRATION OF THE NOVEL ANXIOLYTIC GEPIRONE LEADS TO CHANGES IN HIPPOCAMPAL EPSP ACTIVITY STUDIED IN VITRO. M.H. O'Regan* and J.D. Kocsis. Depts. Neurology and Pharmacology, Yale Med. Sch. and VA Med. Ctr., West Haven, CT. 06516.

The novel anxiolytics, buspirone and gepirone, do not exert their actions until about two weeks. In this study we examined the effects of chronic gepirone administration on synaptic transmission in the CA1 region of rat hippocampus, and compared the acute effects of buspirone and serotonin. Mini-osmotic pumps were loaded with gepirone or with saline and implanted into the peritoneal cavity to deliver 10 mg/kg/day. After 13 to 18 days, hippocampal slices were prepared for electrophysiological study using standard procedures. In slices obtained from rats treated with chronic gepirone there was a reduction in frequency-dependent potentiation of both the presynaptic and postsynaptic components of the field potential. In control slices, bath applied buspirone (100 μM) led to a distinct reduction in the EPSP elicited by stimulation of stratum radiatum, as well as a reduction in presynaptic axonal excitability. Following chronic treatment with gepirone, the effects of acute buspirone application on both presynaptic and postsynaptic potentials were significantly reduced. Additionally, while serotonin (100 μM) had minimal effect on the postsynaptic potential and no effect on the presynaptic fiber volley in control slices, chronic gepirone treatment resulted in serotonin-evoked depression of the EPSP.

These results indicate that chronic treatment with gepirone reduces synaptic facilitation and leads to changes in the acute effects of buspirone and serotonin on synaptic transmission in the hippocampal slice. This implies that the delayed effects of chronic buspirone or gepirone treatment with respect to their anxiolytic action may be retained in the in vitro slice, thereby providing a system to study the long-term modulatory action of these agents on a cellular level.

473.2

REINFORCING EFFECTS OF THE SEROTONIN RECEPTOR AGONIST 8-OH-DPAT:INVOLVEMENT OF THE NUCLEUS ACCUMBENS. T.S. Shippenberg*, C. Stein and A. Herz*. Dept. Neuropharmacology, Max-Planck Inst. for Psychiatry, D-8033 Martinsried, FRG

Place preference conditioning and in-vivo microdialysis were used to examine the motivational effects of the serotonin (5-HT) agonist 8-OH-DPAT and the neural substrates mediating such effects. Rats showed a marked place preference in response to 0.1-0.25 mg/kg 8-OH-DPAT. Other doses were without effect. The 5-HT1a receptor antagonist spiperone or 5,7 dihydroxytryptamine lesions of the n. accumbens (NAC) abolished the effects of 8-OH-DPAT. D-1 but not D-2 dopamine (DA) receptor blockade also abolished 8-OH-DPAT place conditioning.Microdialysis studies revealed a marked decrease in 5-HIAA overflow in the NAC following 0.25 mg/kg 8-OH-DPAT.A slight but significant later-onset inhibition of NAC DA release was also observed. These data demonstrate that 8-OH-DPAT is a secondary reinforcer and that this effect involves both 5-HT1a and D-1 DA receptors. Furthermore, they suggest an important role of the NAC and the 5-HT fibers projecting therein in the mediation of this effect.

Supported by the DFG and Bundesgesundheitsamt.

473.3

DOSE RELATED EFFECTS OF PRENATAL 5-METHOXYTRYPTAMINE (5-MT) ON DEVELOPMENT OF SEROTONIN TERMINAL DENSITY AND BEHAVIOR. A.V. SHEMER, E.C.AZMITIA and P.M.WHITAKER-AZMITIA. Dept. Psychiatry, SUNY, Stonybrook, N.Y. & Dept. Biology, N.Y.University, N.Y.

We have previously shown that 5-MT has a biphasic effect on development of serotonergic neurons in culture and the present study was carried out to determine if this was also observable in vivo. Pregnant Sprague-Dawley rats were injected s.c. with 0.1, 1 or 3 mg/kg 5-MT from gestational day 12 through birth. Terminal density was determined at postnatal day (PD) 1,15 & 30 by measuring uptake of ^{3}H-5-HT. Serotonergic behaviors were tested at PD 5, 15 & 30. The lowest dose of 5-MT produced no change in terminal density at any timepoint, but did produce significant behavioral changes at PD 30. The medium dose produced both a decrease in terminal density and behavioral changes at all timepoints. The highest dose produced an increase in terminal density at all timepoints, but behavioral changes only at early timepoints. These results confirm our findings on development of terminals and also suggest a role for serotonin in " receptor programming " and subsequent serotonin behavioral sensitivity.

473.4

IMPAIRED 5HT FUNCTION AND PROLACTIN RESPONSE TO 5HT AGONISTS IN THE RHESUS MONKEY. G.R. Heninger, S. Evans*, and H. Landis*. Ribicoff Research Facilities, Yale. Univ. Sch. of Med., Conn. Mental Health Ctr., New Haven, Ct 06510.

Depressed patients have a blunted prolactin (PRO) response to i.v. tryptophan (TRP). A 90% reduction in plasma TRP following a low TRP diet and a TRP-free amino acid drink (AAD) produces a return of symptoms in 2/3 of depressed patients recently remitted on antidepressant medication. To evaluate the role of 5HT in the blunted PRO release and the mechanism by which TRP depletion reverses antidepressant effects, the effects of TRP depletion on the PRO response to 5HT agonists was compared to the effects of the 5HT synthesis inhibitor para-chlorophenyalanine (PCPA). Adult male chair trained rhesus monkeys received i.v. TRP at 200 mg/kg or the 5HT1A agonist i.v. gepirone (GEP) at .25 mg/kg to stimulate PRO release. The AAD consisted of 15 essential amino acids with or without TRP added and was administered at 1.5 gm/kg through oral tube, as was PCPA. The TRP and GEP infusions began 4 hours following the AAD and PRO values were measured before and during the infusion with standard RIA methods. The AAD minus TRP produced an average of approximately 60-80% reduction in plasma TRP, along with an 80% reduction of PRO response to infused TRP, and a 40-80% reduction of PRO response to GEP. The AAD with TRP added also reduced the PRO response to TRP and GEP but to a lesser degree, 60 and 30% respectively. PCPA at a total of 300 mg/kg over a 6 day period produced 20 to 40% reduction in the PRO response to TRP and a 25 to 200% increase in PRO response to GEP. The blunted PRO response to TRP following TRP depletion and PCPA and the augmented response to GEP following PCPA are consistent with prior data. The blunted PRO response to GEP following TRP depletion and the blunted response to both TRP and GEP following the TRP augmented AAD suggest that the metabolic changes induced by the amino acid load cause more complex alterations in 5HT function than those observed following the more selective 5HT synthesis inhibitor PCPA. Supported by MH36229 and MH25642.

473.5

SECONDARY CHANGES IN THE EVOKED RELEASE OF SEROTONIN FROM FRONTOCORTICAL NERVE TERMINALS INDUCED BY FLUOXETINE, A SEROTONIN REUPTAKE BLOCKER. A.M.Gardier and R.J.Wurtman. Dept. of Brain and Cognitive Sciences, MIT, Cambridge, MA, 02139.

Previous studies in our laboratory have suggested that the evoked release of serotonin (5-HT) was diminished in rats after chronic administration of fluoxetine, a 5-HT uptake blocker, or d-fenfluramine (d-fen), a drug which both releases 5-HT and blocks its reuptake. However, the treatment then chosen to evoke the 5-HT release was a drug, d-fen which must itself be taken up by the 5-HT uptake system in order to exert its effect. Thus, we decided to evoke 5-HT release by depolarizing 5-HT terminals with a high concentration of KCl, a process that is undependent of 5-HT uptake. We gave rats saline (2ml/kg), fluoxetine (30mg/kg), or d-fen (7.5mg/kg) i.p. for 3 days and measured frontocortical 5-HT release in anesthetized rats (using brain in vivo microdialysis) 24 hours after the last dose. After extracellular frontocortical 5-HT levels stabilized, KCl 120mM was added to the perfusion fluid for 15 min. All samples collected for 15 min. were assayed for 5-HT and 5-HIAA using LC-ECD. Chronic fluoxetine decreased basal 5-HT and 5-HIAA release while chronic d-fen decreased 5-HIAA efflux only. Unlike the situation in d-fen pretreated rats, KCl-evoked 5-HT release was suppressed following fluoxetine pretreatment. This blockade of evoked 5-HT release, and of basal 5-HT and 5-HIAA release were not dependent on the dose of fluoxetine studied, appearing after 7.5, 15, 30 or 60mg/kg. Although 5-HT and 5-HIAA release ultimately returned to normal, blockade did persist after a drug washout period of 7 days. These findings suggest that, unlike d-fen, fluoxetine can cause prolonged impairements in evoked 5-HT release.

473.6

STUDIES WITH NEUROTOXIC AND NON-NEUROTOXIC ANALOGUES OF 3,4-METHYLENEDIOXYMETHAMPHETAMINE (MDMA). M. P. Johnson and D. E. Nichols. Depts. of Pharmacology and Toxicology, and Medicinal Chemistry and Pharmacognosy, School of Pharmacy and Pharmacal Sciences, Purdue Univ., W. Lafayette, IN 47907.

It has previously been reported that rigidification of the α-side chain of *p*-chloroamphetamine (PCA) to give 6-chloro-2-aminotetralin (6-CAT) attenuates the serotonergic neurotoxicity of the parent compound (Fuller, R. W., et al., Arch. Int. Pharmacodyn. Ther. 212:141, 1974). This led us to the synthesis of rigid analogues of MDMA that retained the behavioral activity but lacked the apparent long-term neurotoxicity of the parent compound (Nichols, D. E., et al., J. Med. Chem. 33:703, 1990). One of these rigid analogues is 5,6-methylenedioxy-2-aminoindan (MDAI). The levels of monoamines at short time periods after high doses of MDMA and MDAI were examined. In the frontal cortex and caudate of rats, there are significant differences in the effects on DA and its metabolites with the neurotoxic and non-neurotoxic amphetamine analogues.

This report also describes several additional analogues of MDMA as well as short-term and long-term effects of these analogues. For example, the importance of ring substitution was investigated by examining 3-methoxy-4-methylamphetamine (MMA) and 5-methoxy-6-methyl-2-aminoindan (MMAI). The serotonergic neurotoxicity of these compounds was determined by examining ^{3}H-paroxetine binding and monoamine levels one week following acute doses of 10 or 20 mg/kg s.c. or two weeks following subacute dosing (2x/day for 4 days) of 20 mg/kg. None of the treatments led to changes in serotonergic markers with either MMA or MMAI. These neurotoxic and non-neurotoxic substituted amphetamines were examined for their relative potencies in a number of bioassays. The relative ability of the amphetamines to release and to inhibit the uptake of ^{3}H-5-HT, ^{3}H-DA and ^{3}H-NE was examined. The results will be discussed in terms of the relative importance of these short-term actions on the long-term effects of certain substituted amphetamines.

This work was support by USPHS grant DA-04758 from NIDA.

473.7

IMMUNOCYTOCHEMICAL DEMONSTRATION OF THE ABSENCE OF LONG LASTING EFFECTS OF D-FENFLURAMINE ON NEOCORTICAL SEROTONERGIC AXONS: EVIDENCE AGAINST NEUROTOXICITY. M. Kalia and L. S. Miller*. Dept. Pharmacology, Jefferson Medical College, Philadelphia, PA 19107.

Parenteral administration of D-fenfluramine to rats in massive doses (20-80 mg/kg/day for 4 days) has been reported to selectively remove 5HT-immunoreactivity from axons in the neocortex and striatum up to 4 weeks post-treatment *(Molliver and Molliver, Soc. Neurosc. Abst. 15:210,1988, Molliver and Molliver, Brain Res. 511:165,1990)*. Since these results have significant implications, we repeated this study using D-fenfluramine in **orally** administered (gavage) doses ranging from 2-48 mg/kg/day for 4 days with post-treatment survival periods up to 2 weeks. 5HT-immunoreactive axons were examined in serial, sagittal, 30 um sections in identical regions of the neocortex of D-fenfluramine-treated rats and pair-fed controls. At 24 hrs post-treatment, a progressive, dose-dependent reduction in 5HT-immunoreactive axons in the neocortex was observed. Doses of 12 mg/kg/day for 4 days produced the first evidence of abnormal 5HT-immunoreactive profiles. This dose (12 mg/kg/day for 4 days) and adjacent doses (8 & 16 mg/kg/day for 4 days) were used for the 2 week post-treatment study. Under these conditions neocortical 5HT-immunoreactive axons in the treated animals were comparable to controls. These results demonstrate that D-fenfluramine does not produce permanent loss of 5HT immunoreactive axons in the rat neocortex. Furthermore, the initial loss of 5HT-immunoreactivity does not appear to reflect neuropathology since the immunoreactivity reappears at 2 weeks post-treatment with a pattern similar to that in controls.

473.8

CLONING AND CHARACTERIZATION OF cDNA ENCODING TRYPTOPHAN HYDROXYLASE FROM CENTRAL SEROTONERGIC NEURONS. K. S. Kim, T. Wessel, D.M. Stone, C. Carver, T.H. Joh and D.H. Park. Lab. Mol. Neurobiol., Cornell Univ. Med. Coll. at Burke Rehab. Center, White Plains, NY 10605.

Tryptophan hydroxylase (TPH), the first enzyme in serotonin biosynthesis, is expressed in dorsal raphe nucleus (DRN) and pineal gland (PG). Although higher activity is found in DRN, the levels of mRNA are much greater in the PG. Moreover, biochemical characteristics, such as pI, indicate that different forms may be expressed in DRN and PG. These data raise the question as to whether different forms of TPH exist in these two tissues. To address this question, we analyzed TPH cDNA's by polymerase chain reaction (PCR) using poly A^{+} mRNA purified from both tissues. Several combinations of oligonucleotide primers representing different regions of the coding sequence of pineal gland TPH (Darmon et al., J. Neurochem., 51, 312, 1988) were employed to amplify the TPH signal. The PCR and the subsequent analyses by gel electrophoresis and southern blotting indicated that the same form of TPH message exists in both tissues. Further, the nucleotide sequence of three independent isolates containing the full length coding region was identical to that of PG. In situ hybridization in the DRN indicates that the PG form also is present in the central nervous system. However, TPH mRNA was not detectable in DRN by Northern blot probably due to the low abundance in this tissue. Our results suggest that primary structure cannot alone account for the different behavior and regulation of TPH in the DRN and PG. These experiments do not exclude the possibility that an additional form of TPH not sharing the sequences selected with oligonucleotides is present in the DRN; however, the data clearly indicate that the PG form is expressed in the DRN. Supported by MH44043.

473.9

SEROTONIN IS A SPECIFIC INDUCER OF MOVEMENTS IN UNHATCHED ATLANTIC SALMON (Salmo salar) LARVAE.
B.T. WALTHER, J.V. HELVIK*, D. OPPEN-BERNTSEN* & C. RONG.* Dept. of Biochemistry, Univ. of Bergen, N-5009 Norway.

Exogeneous neurochemicals advance or delay the time of hatching of salmon eggs (Oppen-Berntsen et al.,1990, AQUACULTURE 88, in press). After hatching light induces salmon larvae to initiate bursts of swimming. We tested a number of neurochemicals (40 mg/l) and found that only serotonin caused immediate and recurrent increases in movements of larvae inside eggs posed for normal hatching in daylight. Serotonin induced movements in all larvae after a lag in individual larvae of from a few secs to 15 min at 4 C. Stimulation lasted about 30 min at 4 C, and about 60 min on ice. While normal movements of larvae involve slight rocking or at most a full rotation, serotonin induced 2-5 vigorous rotations. On average control larvae initiate such motions 1.1 times per hour. Serotonin induces a 26.7 fold increase in (more vigorous) rotation motions, while tyramine, dopamine, noradrenalin, adrenalin, histamine and melatonin had no effect. Weak stimulatory compounds were found: tryptamine gave 3.0x increase; N-acetyl-serotonin and 5-hydroxytryptophane both 2.2x increases over control. In these salmon eggs close to term only tyramine appeared to accelerate hatching.Control and treated eggs all had hatched 24 hours after our test, except the adrenalin-treated eggs. Stimulation of hatching movements in salmon larvae is distinct from stimulation of hatching.
Supported by NFFR & Nordic Industrial Fund.

474.1

ALTERATION OF NEURONAL REGULATION OF ASTROCYTOMA PROLIFERATION BY INSERTIONAL MUTAGENESIS. R.A.A. TORRES, R.K.H. LIEM AND M.L. SHELANSKI: COLLEGE OF PHYSICIANS AND SURGEONS OF COLUMBIA UNIVERSITY, NEW YORK CITY,N.Y.10032

It has been previously shown that neuron-astrocyte cell contact results in the arrest of astrocyte DNA synthesis and that this inhibition is membrane-mediated (Hatten, M.E., J. Cell Biol. 104: 1353,1987). In addition, cerebellar granule cell membranes will also inhibit the growth of astrocytoma cell lines derived from rodent and man (Hatten, M.E. and Shelanski, M.L., J. Neurosci. 8: 1447, 1988). In the current experiments we used the retroviral shuttle vector pZIPNEOSV(X) to create a selectable alteration (insertional mutation) in a mouse astrocytoma cell line (G2624), which normally shows growth inhibition in response to granule cell membranes. Successfully infected G-2624 mouse astrocytoma cells were isolated by G-418 selection. These were then again selected for resistance to growth inhibition both by intact neurons and neuronal membrane preparations. Of several hundred G-418 resistant clones that were isolated, one, RM-31 shows significant resistance to neuronal inhibition of DNA synthesis. Indirect immunofluorescent staining for GFAP is strongly positive in both wild-type G-2624 and clone RM-31, with wild-type astrocytic morphology preserved. Quantitation of phenotypic effects by thymidine incorporation and by cell count show that RM-31 has an increased growth rate (1.5 X wild type) and that neuronal inhibition is reduced to 15% that of the wild-type G-2624. ^{35} S-Methionine labeling and extraction shows a decrease or absence of proteins at 210, 110 and 90 kd. Southern blot analysis confirmed viral insertion. The results suggest that we have isolated an astrocytoma mutant in which regions of DNA, normally encoding for proteins which mediate the neuronal regulation of astrocyte DNA synthesis have been altered or disrupted. This lesion could either be at the receptor for the neuronal membrane or at any point along the complex cascade of events regulating cellular proliferation. We are currently in the process of sequencing and amplifying the sequences which flank the proviral genome by inverse Polymerase Chain Reaction (Supported by NS21457).

474.2

EGF- AND TGFα-RESPONSIVE STRIATAL EMBRYONIC PROGENITOR CELLS PRODUCE BOTH NEURONS AND ASTROCYTES. B.A. Reynolds, W. Tetzlaff and S. Weiss. Neuroscience Research Group, Univ. of Calgary, Calgary, Canada.

The effects of EGF or TGFα (15-20 ng/ml) on embryonic CNS progenitor cells were tested by plating E14 mouse striatal cells (1250 cells/cm^2) on poly-l-ornithine coated coverslips in defined, serum-free media. Division of precursors (1-3 cells/cm^2) was first observed at 5-7 days in vitro (DIV); by 14 DIV, these dividing precursors formed proliferating clusters. Untreated cultures showed no such proliferation. Indirect immunocytochemistry with neuron specific enolase (NSE) and glial fibrillary acidic protein (GFAP) was utilized to identify neurons and astrocytes, respectively. At 14-21 DIV, NSE- and GFAP-immunoreactive (IR) cells were found in the proliferating core with some IR cells migrating from the centre. After 21-35 DIV, a large number of cells were GFAP-IR and stellate in appearance; many of the GFAP- and NSE-IR cells had migrated from the proliferating core. While the majority of cells were GFAP- and NSE-IR, a large number of rounded cells with multipolar morphology were negative for both markers. EGF and TGFα effects were similar, yet in TGFα-treated cultures the NSE-IR cells displayed elaborate processes and non-stellate GFAP-IR cells were present. These findings demonstrate that EGF and TGFα induce multipotential CNS precursors in vitro to produce both neurons and astrocytes.

Supported by the Medical Research Council of Canada.

474.3

POSTEMBRYONIC NEUROGENESIS IN THE BRAIN OF *MANDUCA SEXTA*. K.A. Sorensen, N.T. Davis, and J.G. Hildebrand. ARL Division of Neurobiology, University of Arizona, Tucson, AZ 85721.

The nervous system of insects that undergo complete metamorphosis is dramatically reorganized in the transition from larva to adult. One aspect of this reorganization is the postembryonic addition of new neurons to the embryonically derived larval nervous system by divisions of neural stem cells termed neuroblasts [*Wilhelm Roux' Arch.* **164**:247 (1970); *J. Comp. Neurol.* 255:548 (1987); *Dev. Biol.* 125:145 (1988)]. We are studying the origins of the neurons of the primary olfactory center, the antennal lobe (AL), in the brain of the adult sphinx moth *Manduca sexta*. To begin to determine the contribution of postembryonic neurogenesis to the formation of the AL, we used the thymidine analog 5-bromo-2'-deoxyuridine (BrdU), which selectively labels the genome of mitotic cells, to reveal proliferative neuroblasts and the time course of their appearance.

Incorporation of BrdU was achieved by feeding larvae normal diet in which BrdU had been dissolved (0.5 mg/ml). The incorporated nucleotide was then visualized at successive stages of development by means of whole-mount immunocytochemistry employing anti-BrdU antibody and PAP. Scattered neuroblasts were first detected at mid-first instar, with successive increases in the level of staining over the course of the remaining stadia. Labeling is seen first in the brain and subsequently in the subesophageal ganglion and segmental ganglia, developing in an apparently anterior-to-posterior temporal gradient. Staining appears in all ganglia by the beginning of the second larval instar. Whole-mount preparations reveal 3-7 putative neuroblasts that lie close to the larval antennal center and thus may contribute to its metamorphosis into the adult AL. We will use sectioned preparations and cell lineage tracing techniques to ascertain cellular relationships and fates.

474.4

NEUROGENESIS OF THE AMYGDALOID COMPLEX IN THE RHESUS MONKEY. P. Piescinski*1,J.H. Kordower1, P. Rakic2,. 1Dept. of Anatomy and Cell Biology, Univ. Illinois Sch. Med.Chicago Ill. 60612; 2Section of Neuroanatomy, Yale Sch. Med. New Haven Ct. 06510, USA.

The present experiment assessed the developmental window during which neurogenesis of the amygdaloid complex takes place in the fetal primate brain. Nine pregnant rhesus monkeys received an injection of tritiated thymidine between embryonic [E] days 27 and E56 of their 165 day gestation. Offspring were sacrificed in the postnatal period and 8 μm coronal brain sections were processed for autoradiography and counterstained with toluidine blue [Rakic, *Science* 183: 425, 1974].

No radiolabeled neurons are present within any amygdaloid nuclei on E27. A few labeled neurons were observed in the case exposed to tritiated thymidine on E30 making the amygdala, concomitant with the magnocellular basal forebrain nuclei, the earliest developing structure in the telencephalon. At E33, significant labelling was principally located within the central and medial amygdaloid nuclei. A few heavily labeled neurons were present within the lateral nuclear groups as well. Significant neurogenesis occurs for all amygdaloid nuclei by E38 with a peak neurogenesis at E43. There are significantly fewer heavily labeled neurons by E48 and neurogenesis is complete by E56. Thus, all neurons that comprise the amygdaloid nuclei are generated within the first third of gestation. Although a pronounced dorsal to ventral gradient of neurogenesis was observed, the counterclockwise rotation of the amygdala, which begins during the second trimester of morphogenesis, suggests that the actual pattern of cellular development occurs across a medial to lateral gradient. [AHAF, NS 25655; JHK, NS 14841 PR].

474.5

CELL CYCLE KINETICS OF THE E14 MURINE CEREBRAL VENTRICULAR ZONE: ESTIMATES BASED UPON S-PHASE LABELING WITH BUdR. T. Takahashi, M. Jacobson*, R. S. Nowakowski and V. S. Caviness, Jr. Dept. of Neurology, Mass General Hospital, Boston, MA 02114 and Dept. of Neuroscience and Cell Biology, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ 08854.

Proliferating cells in S-phase in the cerebral ventricular zone (VZ) of E14 mouse embryos (E0 = day of conception) were labeled cumulatively in utero with BUdR over 12.5 hours. Embryos were harvested at 0.5, 2.0, 3.5, 6.5, 8.0, 9.5 and 12.5 hr following the initiation of labeling. After immersion and overnight fixation in 70% ethanol, the brains were embedded in paraffin, sectioned at 4 μm in the coronal plane, stained immunohistochemically for BUdR and counterstained with basic fuchsin. Labeled and unlabeled nuclei were counted in the dorsal cerebral wall within a 100 μm wide sector of the VZ. The labeling index (LI = labeled/total population) for the VZ increased approximately linearly from a 0.5 hr value of 0.25 (extrapolated to 0.2 at T0) to 1.0 after an estimated interval of 10.9 hr. The growth fraction is thus 100%, which means that all cells of the VZ are proliferating. The linear increase in LI implies that there is only a single proliferative population. A maximum value for the length of the overall cell cycle (Tc) is computed to be 13.6 and S-phase (Ts) 2.7 hr. Since labeled mitoses were observed 2.0 hr after the initiation of labeling, the approximate length for the G2+1/2 M phases was estimated to be 2.0 hr. With the exception of Ts, these values fall within ranges estimated by cumulative ^{3}H-thymidine labeling in rat embryos of comparable age (E16) by Waechter and Jaensch (*Brain Res.* 46: 235-250,1972): Tc = 12.8 - 16.5 hr, Ts = 6.6 hr, T_{G2+M} = 2.5 hr. Among the considerations that might explain the Ts disparity are a substantial underestimation of the numbers of unlabeled nuclei with the ^{3}H-thymidine labeling method or the possibility that the ^{3}H-thymidine had been available to label S-phase cells for a time substantially longer than a few minute "pulse." It may also be due to differences between the two species.

474.6

DIFFERENTIATION OF ONCOGENICALLY ALTERED CHICK NEURORETINAL CELLS BY SUCCINYLATED CONCANAVALIN A. G.M. Seigel and M.F.D. Notter, Department of Neurobiology and Anatomy, University of Rochester Medical School, Rochester NY 14642.

The orderly course of chick retinal cell differentiation was disrupted *in vitro* by infection with a temperature-sensitive mutant of Rous sarcoma virus (LA29). The result was a continuous retinal cell culture (LA29NR) which remained mitotically active at 42°C, yet rapidly adopted a transformed phenotype upon activation of the pp60src oncogene product at 37°C. As further indication of proliferation, LA29NR cells expressed the proto-oncogene c-fos equally at 42°C and 37°C, as shown by immunocytochemistry and western blot analysis.

Highly proliferative LA29NR cells proved refractory to standard differentiating agents such as cAMP, prostaglandin E1, and retinoic acid. In our novel approach, Succinylated Concanavalin A (SCA), a non-toxic derivative of the lectin Concanavalin A, induced dramatic, reversible morphological changes in LA29NR, including neurite outgrowth and increased cell-to-cell adhesion. SCA also effectively inhibited the growth of LA29NR as evidenced by a massive reduction in tritiated thymidine incorporation.

Since there is a precedent for modulation of proto-oncogene expression during SCA-induced growth inhibition of virally transformed fibroblasts, we currently are assessing the status of v-src and c-fos proteins during SCA-induced differentiation of LA29NR cells.

This work was supported, in part, by training grant T32AG00107 (G.M.S.) and EY 05262 from the National Eye Institute.

474.7

A VASOPRESSIN AND OXYTOCIN CONTAINING NUCLEUS IN THE PIG HYPOTHALAMUS THAT SHOWS AN INCREASE IN NEURON NUMBER DURING PUBERTY. F.J.C.M. van Eerdenburg*, P. Poot*, G.J. Molenaar, F. van Leeuwen and D.F. Swaab*. Dept. Functional Morfology, Faculty of Veterinary Science University of Utrecht, Yalelaan 1, 3508 TD Utrecht, The Netherlands.

The hypothalamus has been studied in the pig since the timing of its brain growth spurt in relation to birth and gonadal steroid levels would be comparable to that of human beings. In the present paper the results of a morphometric and immunocytochemical study of a vasopressin and oxytocin containing nucleus in the pig hypothalamus are presented. This nucleus has not been described before. Neuron numbers and volumes were measured at four different ages i.e. 1 day, 7 weeks, 16 weeks and 30 weeks post natally with a computer assisted method. There were no sex differences found at any of the ages studied. At birth an average of 1200 neurons is found, in the first 7 weeks post natally the number decreased to 770. A striking finding was that between 16 and 30 weeks (i.e. during puberty) an increase from 700 to 1800 (260%) neurons is seen. Immunocytochemistry (antibodies were a gift from Dr. A. Hou-Yu, Columbia University, New York) revealed vasopressin and oxytocin immunoreactive neurons. This increase in the number of vasopressin and oxytocin containing neurons in the pig hypothalamus is much later in development than has been reported so far.

474.8

A MOUSE HYPOTHALAMIC CELL LINE (V1) IMMORTALIZED WITH A TEMPERATURE SENSITIVE LARGE T ANTIGEN. **V. Quinones-Jenab, S. Choi-Kwon, S. Farinelli, S. Jenab*, I. Terleckyj, and H. M. Geller, Department of Pharmacology, UMDNJ-Robert Wood Johnson Medical School and Rutgers University, Piscataway, NJ 08854.**

Dissociated cells from embryonic day 14 mouse hypothalamus were infected with a retroviral construct containing the tsa58 variant of the large T antigen of SV40 and the neo gene (Cepko et. al., Cell 37:1053). Southern blotting and single cell cloning techniques demonstrated that the V1 cells are clonal in origin. This cell line gives rise to flat, GFAP+ astrocyte-like cells **and** round neuronal-like cells which express neurofilament protein. Following single cloning experiments both morphologies appeared, suggesting that we have immortalized a multipotential cell. Flow cytometric analysis indicates that the flat cells express typical glial markers including: NCAM, thrombospondin, and tenascin/cytotactin. These cells have low glutamine synthetase activity, but they express both monoamine oxidase (MAO) A and B. The MAO-A activity increased at 39°C. Levels of these cell specific markers varied between permissive (33°C) and restrictive (39°C) temperatures. The neuronal-like cells developed an inward current after switching to 39°C (see Phelan et al.). All of these data suggest that V1 cells are capable of differentiation after oncogene inactivation, suggesting that these cells can be used as a model to study neuronal and glial differentiation.

474.9

USE OF PCR TO IDENTIFY PRE- AND PERINATAL LETHAL MUTANTS: APPLICATION TO HOMOZYGOUS LURCHER MICE. J. M. Soha and K. Herrup. E.K. Shriver Center, Waltham, MA 02254.

Lurcher is an autosomal dominant mutation located on chromosome 6 of the mouse. Heterozygote (+/*Lc*) animals become ataxic during the first postnatal month in association with the loss of 100% of their cerebellar Purkinje cells as well as other presynaptic neurons. The homozygote condition (*Lc*/*Lc*) has never been described and is presumed to be a pre- or perinatal lethal. To identify *Lc*/*Lc* embryos and, later, *Lc*/*Lc* ↔ +/+ chimeras, a molecular assay has been developed. C57BL/6 and AKR/J mice exhibit a HinFI restriction fragment length polymorphism in the Ig-J_K locus about 2 centiMorgans from the *Lc* locus. AKR/J males were crossed with C57BL/6-+/*Lc* females and the F1 +/*Lc* mice were intercrossed. In the resulting F2 generation, PCR amplification of a 820 bp fragment around the HinFI site, followed by HinFI digestion, lead to a positive identification of any fetus or newborn pup (with about 96% certainty). Homozygous (*Lc*/*Lc*) animals have only the C57BL/6 form of the polymorphism, wild type (+/+) have only the AKR/J form, while heterozygous (+/*Lc*) animals have both.

At E18, *Lc*/*Lc* embryos identified by this method exhibit cerebella that are clearly smaller in cross-section in both the sagittal and coronal planes compared to either +/*Lc* or wild type embryos. Cerebellar volume is decreased by about 40%. Development of folia is also severely retarded. In addition, a greater incidence of pycnotic profiles is seen among cells of the cortical plate in the *Lc*/*Lc* embryos.

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474.10

ROLE OF ELECTRICAL ACTIVITY IN THE DEVELOPMENT AND SURVIVAL OF CULTURED HYPOTHALAMIC NEURONS. D.S.F. Ling, R.E. Petroski, and H.M. Geller. Departments of Pharmacology and Biomedical Engineering, UMDNJ-Robert Wood Johnson Medical School and Rutgers University, Piscataway, NJ 08854.

Hypothalamic neurons exhibit a gradual, consistent increase in spontaneous action potential discharge in long-term culture. To examine the role of electrical activity on neuronal bioelectrical development and survival, we have examined the effects of tetrodotoxin (TTX) and high KCl on cultured hypothalamic cells.

Dissociated neurons from E17 rat hypothalami were plated onto a layer of cortical astrocytes in either microwell trays or on glass coverslips. Starting at 1 day *in vitro*, cultures were treated with TTX and/or high KCl. Neuronal survival was assessed by counting cells on an inverted phase microscope. The development of spontaneous action potentials was examined using an extracellular loose patch recording technique. Cell counting and electrophysiological experiments revealed that TTX caused a marked reduction in both cell survival and the percentage of spontaneously active cells as compared to controls. Concurrent treatment with high KCl inhibits the deleterious effects of TTX on cell survival and the development of spontaneous activity.

When taken together, the results of these experiments suggest that the suppression of electrical activity selectively increases the death of spontaneously active hypothalamic neurons and that the long-term survival of spontaneously active cells may depend on continual membrane depolarization. (Supported by NIH NS 25168)

474.11

SELECTIVE DEGENERATION OF LARVAL THORACIC MUSCLE BY KILLER-CELLS DURING METAMORPHOSIS IN DROSOPHILA. W.J. Costello. Basic Sciences/COM & Dept. Zool./Biomed. Sci., Ohio University, Athens, OH 45701.

Holometabolous insects must undergo complete reorganization to change from larval form to adult form. Hormone release, temporally and quantitatively, plays an integral role in controlling this complex process. Larval muscles and neurons respond to varying levels of specific hormones; degeneration of these tissues occurs in response via programmed cell death. I have observed a large (6X13 um), unique cell type appearing in the thoracic region during early metamorphosis in Drosophila which plays a role in muscle degeneration. These cells become prevalent in the early prepupa (5h), first appearing at ventral sites and then migrating dorsally along body wall muscles. Each cell attaches to a muscle prior to any sign of degeneration in the muscle itself. Subsequently, the cell extends processes into the muscle and degeneration of the muscle rapidly results. This event occurs progressively from the prothoracic region to the metathoracic region. The migration of these cells to the muscles happens through an active identification process. Although most thoracic bodywall muscles are attacked by the "killer-cells", a few adjacent muscles lack the cells. These persistent muscles serve as substrates for adult myocytes which will form the adult indirect flight muscles.

Such "killer-cells" appear to be another important factor in programmed cell death of muscles during metamorphosis. Their ability to avoid selectively the persistent larval muscles implies a recognition process by these cells. The basis of this selection and the hormonal control of these cells' activity are being studied.

475.1

MARKERS OF NEURONAL MATURATION IN NEUROBLASTOMA CELLS TREATED WITH NEOCARZINOSTATIN. N. F. Schor, M. R. Gilbert*, J. Lowengrub*, and C. F. Lagenaur, Children's Hospital of Pittsburgh, Pittsburgh, PA. 15213, The Johns Hopkins Hospital, Baltimore, MD. 21205, and University of Pittsburgh, Pittsburgh, PA. 15261

Treatment of murine neuroblastoma cells with the antineoplastic natural product neocarzinostatin results in morphologic differentiation. The cells extend processes, stop dividing, and, unlike their "immortal" untreated counterparts, die after 14 days in culture. We have looked at these cells for the presence of several known chemical markers of neuronal maturation to better define the effects of neocarzinostatin upon them. Cells treated with neocarzinostatin for 1 hour on day 0 express nerve cell adhesion molecule (N-CAM) by day 3 in culture, as shown by bright cell surface staining with fluorescent polyclonal antibodies to N-CAM; untreated cells do not stain for N-CAM. Staining with antibodies directed against L-1 or HNK-1 demonstrates no change in these antigens upon treatment of neuroblastoma cells with neocarzinostatin. Both L1 and HNK-1 are cell adhesion molecules which share a common carbohydrate epitope with N-CAM. Electron microscopy, histochemical staining, and Northern blotting indicate that neurofilaments are not present in treated or untreated cells. No change in the amount of mRNA for GAP 43 is found by Northern blotting at 24, 48, 72, or 96 hours after treatment of neuroblastoma cells with neocarzinostatin; this protein is normally associated with axonal outgrowth. These studies suggest that the processes extended by neuroblastoma cells are not normal axons, and that the morphologic differentiation induced by neocarzinostatin is not indicative of "normalization" of neuroblastoma cells.

475.2

IMMORTAL CELL LINES FROM EMBRYONIC RAT HIPPOCAMPAL PRECURSOR CELLS . E.M.Eves[1], H.J.Lee[3], M.S.Tucker[1], M.R. Rosner*[1] and B.H. Wainer[1,2,3]. Depts.[1]Pharm. & Phys. Sci., [2]Path. and [3]Comm. on Neurobio., The University of Chicago, Chicago, IL 60637.

Temperature-sensitive alleles of the SV-40 large T-antigen have been used to immortalize hippocampal cells from embryonic day 17 (E17) and E18 rats. The resultant cell lines proliferate at the permissive temperature (33°); at the non-permissive temperature (39°) proliferation ceases and the cells undergo morphological changes. A lowered concentration of fetal calf serum (1%) at 39° induces the formation of cellular processes in many of the lines; in some, these appear neuritic and this morphological differentiation is potentiated by phorbol ester. Immunostaining for neurofilament proteins (NFP) and glial fibrillary acidic protein (GFAP) reveals several phenotypes. Of 12 lines examined, 7 are NFP+ GFAP-, 2 are NFP- GFAP+, and 3 have both NFP+ and GFAP+ cells indicating that the immortalized lines include progenitor types not yet committed to a neuronal or a glial pathway, as well as already committed cells. Microtubule-associated protein 2 (MAP2) and growth-associated protein (GAP43) have been visualized by immunostaining in several NFP+ lines. The former has been reported to be present in the dendrites and the latter in the axons of mature neurons. In developing neurons both are distributed throughout the cell. In our cell lines these markers tend to be assymetrically distributed in the perinuclear region except in the most differentiated (by morphology) cells. Since the proportion of cells undergoing morphological differentiation and the combinations of the listed markers vary from line to line it is likely that we have immortalized several distinct types of hippocampal precursor cells. (Supported by NS-25787 and the Alzheimer's Disease and Related Diseases Assn.)

475.3

EXPRESSION OF EPIDERMAL GROWTH FACTOR RECEPTOR IN IMMORTALIZED RAT HIPPOCAMPAL CELL LINES. M.S. Tucker[1], E.M. Eves[1], X.Y. Hou[2*], B.H. Wainer[1,3,4], and M.R. Rosner[1*]. Depts. [1]Pharm. & Phys. Sci., [2]Mol. Gen. & Cell Bio., [3]Path. and [4]Comm. on Neurobiology, The University of Chicago, Chicago, IL 60637.

Epidermal growth factor (EGF) is a potent mitogen for fibroblast and epithelial cells. In order to characterize the EGF receptor in a nonproliferating cell system, we utilized hippocampal cell lines capable of terminal differentiation. We had previously shown expression of EGF receptor in primary hippocampal cultures by immunostaining. After cell line differentiation, serum failed to stimulate DNA synthesis, although the retraction of cell processes was observed. We screened several of the cell lines for EGF receptor by binding EGF before and after differentiation. One line, WH19-4, exhibited a 20-fold decrease in binding activity while another line, H19-7, increased its binding activity 8-fold after differentiation. Experiments in which these two cell lines were transfected with the EGF receptor promoter suggest that the changes in EGF receptor binding result from changes in receptor transcription. Thus, these two hippocampal cell lines provide powerful tools for studying both positive and negative regulation of growth factor receptor transcription during hippocampal cell development, as well as allowing us to investigate nonmitogenic cell signalling pathways by the EGF receptor tyrosine kinase. (Supported by 5 T32 GM 07151-14 and International Life Sciences Institute.)

475.4

CELLULAR INTERACTIONS BETWEEN SCHWANN CELLS AND PC12 CELLS *IN VITRO*. J.-H. Tao-Cheng, O. Okuda*, D. L. Simpson*, L. Chang* and M. W. Brightman, Lab. of Neurobiology, NINDS/NIH, Bethesda, MD

A rat pheochromocytoma cell line, PC12, was co-cultured with dissociated Schwann cells (SC) from sciatic nerves of 2 day old rats. SC were usually seeded first. They were spindle shaped and formed clusters of closely spaced cell bodies with processes radiating outward. Naive PC12 cells, when added, randomly settled initially, then gradually migrated toward the clusters of SC. By 3-4 days, 60-80% of the PC12 cells were aggregated on top of the SC. Differentiated PC12 cells, e. g., nerve growth factor NGF-primed or K-ras virus infected, exhibited this affinity toward SC even earlier: by 24-36 hrs in co-culture. There was no aggregation of PC12 cells over contaminating fibroblasts in the SC preparations, or over other types of control cells, such as astrocytes, brain and aortic endothelial cells. The close PC12-SC association consisted of PC12 cells stacked, sometimes 5-8 cells deep, on top of SC. Ultrastructurally, in solo SC cultures, the cells usually contained many more intermediate filaments (IF) than did SC *in vivo*. Sometimes, the IF were packed in crystalline form in the cell body. Microtubules were prominent in the cell processes. In co-culture with PC12 cells, some SC underwent differentiation: an increase of rough endoplasmic reticulum, a decrease of intermediate filaments and ensheathment of PC12 neurites by flat SC processes. The wrapping of PC12 neurites by sciatic nerve SC is in contrast to the report of non-ensheathment by SC from dorsal root ganglia. Conversely, both SC and SC derived extracellular matrix supported neurite extension from PC12 cells. Theses neurites contained abundant microtubules, dense core granules (100 nm) and small, clear vesicles (50 nm). In these co-cultures, the production of choline acetyltransferase, assessed biochemically, increased by 50-100% while that of acetylcholinesterase was largely unchanged.

475.5

CHARACTERIZATION OF BI-LAMINAR CULTURES FROM E16 RAT SEPTUM. J.D. Zucker, M.V. Jones, N.L. Harrison, H.J. Lee, J.D. Roback and B.H. Wainer. University of Chicago, Chicago, IL 60637.

The bi-laminar culture system, developed by Banker and Cowan in the hippocampus, allows for isolation of pure neurons at low density and selective enrichment for neuronal sub-populations depending on age of embryonic tissue. In the current study, such cultures derived from E16 rat septum have been established and partially characterized. Glia derived from P2-P3 rat cortices are grown to confluence in 30-65mm culture wells. E16 rat septal cells are then plated at low density (approx. $5000/cm^2$) on coated glass coverslips which are inverted and supported less than one millimeter above the glial plane by paraplast "feet". The cultures are treated with an antimitotic agent at day 4 to inhibit glial growth, and maintained for up to five weeks in N2.1 medium (serum-free). The cultures have been characterized with respect to cytoskeletal and neurochemical markers. In the glial plane, greater than 99% of cells stain positively for the glial marker glial fibrillary acidic protein (GFAP), with occasional neurons expressing neurofilament proteins (NFP). Conversely, in the neuronal plane, over 99% of the cells stain positively for NFP. Staining for growth associated protein GAP-43 and microtubule associated protein MAP-2 reveals neuritic specialization into axons and dendrites. Immunostaining for the neurochemical markers choline acetyl-transferase (ChAT), g-aminobutyric acid (GABA), and nerve growth factor receptor (NGFr) was performed on cultures grown in the presence or absence of NGF. In cultures grown in the absence of NGF, the proportion of ChAT-positive cells is less than 1%, while 65±4% stain for GABA and 24±9% stain for the NGF receptor. When cultured in the presence of 100 ng/ml NGF, the number of ChAT-positive cells increases significantly to 11±8%, while GABA and NGFr staining remains unchanged. There is no discernable effect of NGF on morphology or cell survival. The bi-laminar system shows a greater proportion of ChAT-positive cells than previously described monolayer culture systems in the presence of NGF. This initial study provides a baseline neurochemical characterization of E16 septal cultures in the presence and absence of NGF, against which both other time points and other trophic factors may be compared (Supported by NS 25787).

475.6

COMPARISON OF TOTAL CELL PROTEIN PRODUCTION IN ORGANOTYPIC CULTURES FROM MOUSE SPINAL CORD WITH THAT IN INTACT MICE H. L. Stewart, J. A. Birk*, N. A. Mills* and M. H. Droge, Dept. of Biology, Texas Woman's Univ., Denton TX 76204.

The goal of our research is to establish an in vitro mammalian spinal preparation suitable for investigating the mechanisms underlying motor pattern generation. One model system currently under investigation involves organotypic cultures from 300 µm transverse sections of neonatal mouse lumbar spinal tissue. To ascertain relative developmental and maturational indices between the cultured tissue and that of intact mice at similar postnatal ages, the current study compares morphology and total cell protein production in both preparations.

Lumbar spinal tissue obtained from neonatal mice was sliced and established in roller drum culture; fresh, noncultured tissue was similarly obtained and sliced. At similar postnatal intervals, samples from each group were incubated in medium containing ^{35}S-methionine and ^{3}H-thymidine and examined for 1) precipitable counts via scintillation counting, and 2) protein patterns from electrophoresis. Cultured and fresh tissue were stained for identification of cholinergic neurons at day 0 (day of culturing), three weeks, and five weeks. Preliminary data indicate that the total cell protein profile reflects the changes seen in size and density of cholinergic neurons at the sampled intervals tested. (Supported by NIH1R29 NS 25250-01)

475.7

DIFFERENTIAL MODULATION OF PROTEIN KINASE C ISOZYMES IN DIFFERENTIATION OF PC12H CELLS STIMULATED BY DIBUTYRYL C-AMP. S. Shimohama, Y.U. Kunugi*, H. Tamura*, T.Taniguchi*,H.Ninomiya*,J. Kimura* and T. Saitoh. Dept.of Neurology, Kyoto Univ. Sch. of Med.,Dept. of Neurobiology, Kyoto Pharmaceutical Univ., Kyoto 606, Japan., Dept. of Neurosciences, Univ. of California, San Diego, La Jolla, CA 92093,USA.

PKC is a family of closely related enzymes, and the role of each PKC isozyme remains unknown. Rat pheochromocytoma PC12 cells can be induced to differentiate into cells with typical neuronal morphology by a number of substrates, however, the molecular mechanisms are not clear. To clarify the different functional role of each isozyme, we examined the differential modulation of four types of isozymes in the differentiation of PC12h cells by using immunoblotting analyses. We have employed four anti-PKC antisera raised against C-terminal variable region of PKC(α),(βI),(βII), and (γ). Two types of PKC isozymes, α and βII were predominantly detected in PC12h cells. When exposed to 1mM dibutyryl cAMP, PC12h cells clearly began to exhibit the neurite outgrowth at around 30 min. In this stage, neurites looked narrow and unstable. After 24 hours, these neurites became much stable and wide ribbon-like shapes. When immunoblotting was carried out for the homogenates of dibutyryl cAMP-stimulated PC12h cells, we found the prompt and temporary accumulation of PKC(α), whereas the accumulation of PKC(βII) was prolonged for nearly 24 hours following the addition of dibutyryl cAMP. These results suggest that PKC(α) and PKC(βII) may be involved in different cellular processes and regulate the specialized physiological responses in the differentiation of PC12h cells and that concerted action between protein kinase C and cAMP-dependent protein kinase systems may work on the differentiation process of PC12h cells.

475.8

SURVIVAL AND DIFFERENTIATION OF PURIFIED PURKINJE CELLS FROM MOUSE CEREBELLUM C.A. Baptista, M.E. Hatten, and C.A. Mason, Dept. Pathology, College of Physicians and Surgeons, Columbia University, New York, N.Y. 10032

In our studies of axon-target interactions *in vitro* (Baird et al., this volume), we have shown that purified cerebellar granule neurons specifically signal their appropriate afferents, the pontine mossy fibers, to stop growing. To examine afferent growth cone behavior and interactions with Purkinje cells, we have developed affinity methods to purify this cell population. Because Purkinje cells are relatively more numerous in fetal life, we dissociated cerebellum from E15-18 and immunoabsorbed Purkinje cells to plastic dishes previously coated with antibodies against Thy 1.2. Cells were subsequently replated on polylysine-coated dishes. The purity was assessed with an antibody to calbindin (gift of S. Christakos), which is specific for Purkinje cells. Although the yield of Purkinje cells was very high with this method, neurite outgrowth and cell polarity were impaired, and survival was limited to 1-2 days. In contrast, in the presence of glial cells and other neurons, Purkinje cells survived for up to two weeks, and developed long axons and elaborated characteristic dendrites. Studies are in progress to identify the factors (substrates, growth factors, and surface molecules) critical to the survival and differentiation of purified Purkinje cells. This culture system will in turn allow us to test the specificity of axon-target interactions, and the effect of olivary (climbing fiber) afferents on Purkinje cell morphogenesis. Supported by NS 16951.

475.9

BUBBLE-LIKE MINISPHERES OF AVIAN EMBRYONIC NEUROEPITHELIUM: A MODEL FOR STUDYING FLUID TRANSPORT. M. E. Desmond and M.C. Field*. Department of Biology, Villanova University, Villanova, PA 19085.

To date, it is unknown when the neuroepithelium (NE) first becomes secretory in the vertebrate embryo and whether this capability to secrete is ubiquitous initially to all three primary brain vesicles. A method has been devised to culture the primary brain vesicles along with their associated head mesenchyme and skin ectoderm (SE). Embryonic brain explants were cultured in mini-wells containing either Hanks BSS or BSS plus bovine fetal calf serum plus antibiotics. From a total of 224 explants cultured in BSS and 71 explants in enriched medium, it is concluded that fluid -filled mini-spheres form from both SE and NE; these mini-spheres appear identical irrespective of embryonic brain origin; the size of the mini-sphere formed depends on the size of the brain vesicle explant; SE explants form mini-spheres 24-48 hrs earlier than neuroepithelial explants; and mini-spheres form preferentially in enriched medium. In order to clearly distinguish between NE and SE explants, blastodiscs of 70 embryos of neural plate stages (HH stages 4-9) were treated for 20 min at 4^{0} C in 1% crude trypsin, rinsed in 5 washes of BSS, 5 min each and then microsurgically manipulated to separate the neural plate from the skin ectoderm. From nineteen preliminary SE and NE explants cultured in enriched medium, 79% of the SE (after 18-24 hrs) and 32% of the NE explants (after 72 hrs) formed mini-spheres. (Supported by NIH grant No. HD24710).

DIFFERENTIATION, MORPHOGENESIS AND DEVELOPMENT: FOREBRAIN

476.1

PROTO-ONCOGENE PROTEIN EXPRESSION DURING MOUSE BRAIN DEVELOPMENT. J.B. Hutchins. Department of Anatomy, University of Mississippi Medical Center, Jackson, MS 39216-4505.

Proto-oncogenes and their protein products are believed to play a key role in mammalian development. Proteins transcribed from proto-oncogenes are known to regulate the expression of numerous genes, and by this mechanism they may determine a cell's fate.

The pattern of expression of the growth factor PDGF (which can exist in AA, AB or BB dimeric forms) as well as the proto-oncogene proteins Myc and Sis (homologous to platelet-derived growth factor B chain, $PDGF_B$) were studied during prenatal mouse CNS development (embryonic days 10 through 16, E10-E16). All three proteins have been implicated in control of CNS cell lineage.

Expression of PDGF-like immunoreactivity has begun by the earliest time point studied (E10 spinal cord, E12 retina). Long, thin, spike-like processes extend out from these labeled cells in both regions. However, in pros-, mes- and rhombencephalon, $PDGF^+$ cells are still round and clustered in the ventricular zone. Later, $PDGF^+$ cells in all three encephalic regions take on a more differentiated appearance. No appreciable PDGF immunoreactivity is seen in the brain of juvenile or adult animals.

Myc-like immunoreactivity in the developing CNS is not seen until about E15. In the E15 retina, Myc^+ nuclei cluster in the developing ganglion cell layer, near the vitreal surface. No Myc^+ cells are seen in the presumptive outer layers of the retina. Myc^+ nuclei are scattered throughout the spinal cord at E16 in an apparent random distribution.

The pattern of Sis-like immunoreactivity is similar to that of Myc in both time course and distribution. The distribution of Sis^+ cells is strikingly different from cells labeled with the antibody to PDGF. The specificity of the PDGF antiserum is still being explored, but may reveal interesting disparities between the localization of Sis/$PDGF_B$ and the putative distribution of AA or AB dimers. Supported by BRSG RR05386 (to U.Miss.) and EY08228 (to J.B.H.).

476.2

A ROLE FOR Ic IN REGULATING ACTION POTENTIAL DURATION IN HIPPOCAMPAL NEONATAL NEURONS OF RABBIT. J.V. Sanchez-Andres and D.L.Alkon. Laboratory of Molecular & Cellular Neurobiology, NIH, Bethesda, MD 20892.

Duration of the action potentials (AP) in CA1 cells of hippocampus in developing cats is prolonged when compared with adults. This effect has been attributed to the slower rate of repolarization (Purpura et al. 1968). Schwartzkroin (1982) confirmed this observation in newborn rabbits, and observed that the APs showed a duration similar to that of adult cells by about day 8 post partum. Furthermore, Storm (1988) proposed that the last two thirds of the AP repolarization phase are generated by Ic in CA1 neurones.From the above, we propose that Ic is incompletely developed at birth and that this contributes to the long AP duration. We recorded both Ic and I_{AHP} (Ca^{2+} dep-K^+ currents) in CA1 neurones from newborn (1st-8th days after birth) and adult rabbits using a single electrode voltage-clamp (SEVC) method. Semilogarithmic I-V curves for the net Ic current values at the steady state, closely fit straight lines (regression coeff.:M±SE:adult, .98±.01; newborn, .96±.01). The slope values for the neonates were clearly reduced (M±SEx10^{-2}:4.06±.29, n=16) compared with the adults (5.83±.50, n=18), ($p<0.01$, unpaired, two tailed t test). The rising phase of I_{AHP}, usually unobservable in adults, was clear in the neonates, although there were not significant differences in I_{AHP} amplitudes.

These data a) suggest that a small Ic is responsible for the longer duration of the AP in neonates, b) raise the possibility that neonatal CA1 K+ currents are not yet affected by learning-induced reductions as previously demonstrated for adults (Coulter et al., 1988; Sanchez-Andres and Alkon, 1989).

476.3

DEVELOPMENT OF BASAL FOREBRAIN CHOLINERGIC PROJECTIONS TO CEREBRAL CORTEX: ACHE HISTOCHEMICAL STUDIES IN RATS AND HAMSTERS. R.T. Robertson, G.H. Kageyama, K.A. Gallardo* and J. Yu. Departments of Anatomy and Neurobiology and Physical Medicine and Rehabilitation, College of Medicine, University of California, Irvine, CA 92717.

The structure and function of basal forebrain cholinergic systems have attracted a great deal of attention in recent years, but development of these projections is not well understood. We are studying development of basal forebrain projections to cerebral cortex by AChE histochemical techniques.

Normal rat and hamster pups from postnatal day 0 (P0) to P14 were deeply anesthetized and sacrificed by vascular perfusion with aldehydes. Frozen sections cut in the transverse, parasagittal, tangential, or oblique planes were processed for AChE histochemistry, using the techniques of Tago et al (1986) or Koelle and Friedenwald (1949). Adjacent sections were Nissl stained.

AChE-positive axons destined for cerebral cortex appear to travel along either medial or lateral routes. AChE-positive axons traveling the medial route pass rostrally and dorsally from the nucleus of the diagonal band, coursing around or through the genu of the corpus callosum, to reach the cingulate bundle and supracallosal stria. Axons traveling laterally pass from the substantia innominata and medial globus pallidus to gain access to the subcortical white matter. AChE-positive axons can be seen leaving the basal forebrain region by both routes in P0 rats and hamsters. Large numbers of these axons appear to reach the deep layers of frontal cortex by P0, but only a very few have reached the occipital pole by this time. AChE-positive axons appear first in deeper layers of cortex, and grow into more superfical layers as these layers differentiate from the cell dense cortical plate. AChE-positive axons appear to grow very rapidly, and assume adult-like areal and laminar distributions by the third postnatal week.

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476.4

Subplate Cortical Neurons of the Rat: Studies *in vivo & in vitro*. Casey M. Annis, Glenn H. Kageyama, Richard T. Robertson and Jen Yu, Depts. of Anatomy & Neurobiology and Physical Medicine and Rehabilitation, College of Medicine, University of Calif., Irvine, Ca., 92717.

The subplate region of the developing cerebral cortex is believed to play a crucial role in the development of the cortical plate. We report here a broad characterization of the subplate region of the rat, both *in vivo* and *in vitro*. Immunocytochemical, histochemical and (^{3}H)-thymidine autoradiography techniques were used to analyze the development of the subplate region in frozen sections and in organotypic slice cultures (Annis et al., '90) of fetal & newborn rat pups of varying age (E19-P14). (^{3}H)-thymidine autoradiography, in sections of parieto-occiptal cortex, indicates that subplate neurons in the rat are born between E13-14 and an identifiable subplate zone can be detected up to adulthood. Immunocytochemistry with antibodies against GABA, NPY, Somatostatin, and VIP indicate that populations of subplate neurons both *in vivo & in vitro* stain positively with these antisera. Histochemical data indicate that a population of subplate neurons also stain strongly for acetylcholinesterase, *in vivo & in vitro* . These cells are horizontally or vertically oriented bipolar & multipolar neurons. A subpopulation of these AChE positive neurons demonstrate pseudopyramidal morphologies. Comparison of these data with those obtained by other investigators in cat and primate indicate strong similarities between the subplate neurons of the rat and those of other mammals.

Supported by NSF grant 87-08515 and NIH grant NS 25674.

476.5

DEVELOPMENT OF NEUROARCHITECTURE AND SENSITIVITY TO EAA NEUROTOXICITY IN CULTURED HUMAN CEREBRAL CORTICAL NEURONS. B. Rychlik, J.-S. You*, J. E. Sisken* and M. P. Mattson. Center on Aging, Depts. of Anatomy & Neurobiology, Immunology & Microbiology, Univ. of Kentucky, Lexington, 40536.

The development and aging of the human cerebral cortex occurs over a time period greatly prolonged when compared with that of the most commonly studied laboratory animals. In addition, the human brain is vulnerable to developmental (e.g., Downs syndrome) and age-associated (e.g., Alzheimer's) disorders not evident in lower species. We have initiated experiments aimed at understanding the cellular and molecular bases for such species-specific differences. Low density cell cultures of fetal human and rat cerebral cortex were established and maintained under identical conditions. Neurons in the human cultures extended axons at a much slower rate, and lived much longer than did rat neurons. Culture medium exchange experiments indicated that these differences were probably not due to cell-derived factors released into the culture medium. In human neurons, excitatory amino acids (EAAs) elicited calcium responses in a supopulation of neurons at 2 weeks in culture and by 35 days EAA neurotoxicity occurred. Responses to EAAs developed much more rapidly (within 6 days) in the rat neurons. Perturbations of calcium homeostasis demonstrated a superior calcium buffering ability in the human neurons. The slower development and longer in vitro "life span" of the human neurons suggests that species-specific differences in brain development and aging may result from differences in the individual neurons. Further direct comparisons of neurons from long- and short-lived species may provide insight into fundamental mechanisms of neuronal growth and aging. (supported by a PSP grant and an ADRDA Faculty Scholar Award to MM).

476.6

CONNECTIVITY OF FETAL LIMBIC CORTEX TRANSPLANTED INTO NON-LIMBIC CORTEX. M.F. Barbe and P. Levitt, Medical College of Pennsylvania, 3200 Henry Ave., Phila., PA 19129.

Previous studies using a molecular marker of limbic phenotype, in combination with brain tissue transplants, has shown that there is an early critical period for irreversible commitment of developing cortical neurons to exhibit limbic features. The present study was undertaken to determine whether there is also an early specification of projection phenotypes in the limbic or neocortical transplants. Limbic (perirhinal) and nonlimbic (sensorimotor) fetal tissue was transplanted into P1 rat host perirhinal or sensorimotor cortex. Animals were sacrificed 2 weeks later and DiI crystals precipitated onto pieces of finely-pulled glass micropipettes inserted into the transplant area. The brains were incubated at 37^{o}C for 6-7 weeks. Afferent and efferent projections of homotypic and heterotypic transplants were analyzed. DiI labeling of perirhinal cortex transplanted into sensorimotor cortex retrogradely labeled limbic-associated thalamic nuclei (e.g. lateral dorsal, parafascicular), as well as normal somatosensory thalamic nuclei (e.g. VPM, VPL). In addition, DiI-labeled efferents from the transplants could be traced to similar nuclei in the thalamus. Perirhinal transplants into perirhinal regions show exclusively limbic labeling. Sensorimotor transplants into sensorimotor regions did not show any limbic projections. Experiments are in progress to examine heterotypically transplanted sensorimotor into limbic cortex. The data suggest that while molecular specification is under relatively strict regulation, phenotype as defined by projection patterns are subject to more complex environmental influences. Supported by NIH grant MH 45507.

476.7

GROSS MORPHOMETRY OF FRONTAL, PARIETAL, AND TEMPORAL CORTEX IN MONOZYGOTIC TWINS C.E. Thomas*, M.J. Tramo, W.C. Loftus*, C.H. Newton*, and M.S. Gazzaniga Program in Cognitive Neuroscience, Dartmouth-Hitchcock Medical Center, Hanover, NH 03756

We considered the role of genetic influences in the development of the cerebral cortex and regional variation therein by comparing the size and shape of the frontal, parietal, and temporal cortex of two pairs of monozygotic twins using the cortical flat-mapping technique of Jouandet et al. (J Cog Neurosci 1:88-117,1989).

All subjects were young right-handed women. Monozygosity was determined by red blood cell surface marker assays and a standardized questionnaire. Straight-line two-dimensional computer reconstructions of the unfolded cortical surface ("brainprints") were generated from thin-section T1-weighted coronal magnetic resonance images. The per cent surface area overlap of each map was determined by: 1) superimposing two maps of a given lobe aligned at the dorsomedial-most sulcus of that lobe on the brainprint (cingulate sulcus for the frontal and parietal lobes, lateral fissure for the temporal lobes); 2) tracing by hand the outer border of the superimposed maps and the region of overlap within it; 3) measuring by computerized planimetry the surface area contained within the former and the latter; and 4) calculating their per cent overlap. Maps of each lobe were paired within and across twins.

In twin pair A, left frontal, parietal, and temporal cortex showed 81%, 71%, and 79% overlap and the right 82%, 70%, and 83% overlap, respectively. In twin pair B, left frontal, parietal, and temporal cortex showed 75%, 78%, and 82% overlap and the right 79%, 71%, and 76% overlap, respectively. The mean overlap of left frontal, parietal, and temporal cortex of the unrelated twin pairings (N=4) was 82%, 71%, and 81% and of the right 75%, 67%, and 77%, respectively. The mean overlap of all pairings combined (N=12) was 79%, 70%, and 80% for frontal, parietal, and temporal cortex.

These preliminary findings raise the possibility that the gross morphometry of parietal cortex varies more than that of frontal and temporal cortex. No striking difference was observed in twin pairs versus unrelated pairs. [Supported by ONR N00014-89-J-3035 (MJT & MSG) and AFOSR-89-0437 (MSG)]

476.8

BRAINPRINTS: INTER- AND INTRA-OBSERVER RELIABILITY M.L. Jouandet, M.J. Tramo, C.E. Thomas*, C.H. Newton*, W.C. Loftus*, John B. Weaver*, and M.S. Gazzaniga Program in Cognitive Neuroscience, Dartmouth-Hitchcock Medical Center, Hanover, NH 03756 and Dept. of Radiology, The New York Hospital-Cornell Medical Center, NY, NY 10021

We recently described a new in vivo method of unfolding, mapping, and measuring the human cerebral cortex using straight-line two-dimensional computer reconstructions of magnetic resonance images (Jouandet et al. 1989; J Cog Neurosci 1:88-117). In order to consider the error inherent in our reconstruction procedures, inter- and intra-observer reliability were assessed by having four observers independently map one hemisphere and a fifth observer map one hemisphere three times.

Among the four observers, total hemisphere surface area (SA) ranged from 930-1067cm^2, frontal lobe SA 229-249cm^2, parietal lobe SA 219-249cm^2, temporal lobe SA 193-222cm^2, occipital lobe SA 132-145cm^2, and limbic/paralimbic cortical SA 134-160cm^2. As an index of inter-observer reliability, the coefficient of variation (CV) of each region of interest (ROI) SA measurement was calculated by dividing the population standard deviation by the population mean; in addition, pair-wise correlations across ROI SAs for the four observers were calculated. For total hemisphere, frontal, parietal, temporal, and occipital SA, CV was 5.4%, 3.5%, 6.4%, 2.7%, and 15%, respectively. Among the 27 ROIs, SA measurements showed the least variation in the orbitofrontal gyri and pars triangularis (3.0%) and the greatest in the superior lateral occipital gyrus (28%); ROI median CV was 9.4%. Pair-wise correlations ranged from 95.9-99.0%. CVs were also calculated to estimate intra-observer reliability. For total hemisphere, frontal, parietal, temporal, and occipital SA, CV was 2.7%, 2.1%, 3.7%, 4.4%, and 8.3%, respectively.

These results compare favorably with accuracy estimates of regional cortical SA measurements obtained using the contour method of flat-mapping post-mortem animal cortex (Van Essen and Maunsell 1980) and indicate that our brainprinting technique yields reasonably reliable estimates of regional cortical SA in vivo.

Supported by ONR N00014-89-J-3035(MJT & MSG) and AFOSR-89-0437(MSG)

476.9

RADIAL GLIAL IMMUNOREACTIVE FIBERS IN THE REGION OF SPONTANEOUS MICRODYSGENESIS IN NEWBORN NEW ZEALAND BLACK MICE. G.F. Sherman, D.M. Press*, G.D. Rosen, and A.M. Galaburda. Department of Neurology, Harvard Medical School, and Beth Israel Hospital, Boston, MA 02215.

The New Zealand Black mouse strain (NZB) spontaneously develops severe autoimmune disease which produces premature death at about 16 months of age. In adulthood 20-40% of these mice have ectopic collections of neurons in layer I with underlying distortion of the cortical layers (Sherman et al., Acta Neuropathol., 74:239-242, 1985). These ectopic collections most likely are formed during the time of neuronal migration, which extends in the mouse from the late part of gestation to the early postnatal period. In the present study we examined newborn NZB pups with ectopias in layer I for evidence of a disturbance to radial glial fibers.

Newborn NZB pups were transcardially perfused with 4% paraformaldehyde, the brains removed and immersed overnight in paraformaldehyde. The brains were cut coronally at 30μm, every tenth section was stained with thionin, and an adjacent series was stained for radial glial fibers using Rat-401 antibody (provided by S. Hockfield). Thus far, 3 brains with ectopias have been stained.

There was aberrant organization of radial glial fibers focally in the area of the ectopias. Specifically, the external limiting membrane, which was enhanced by antibody staining, was interrupted in the area of the ectopia and it appeared as though neurons had migrated into the space created by the interruption. In addition, a slight increase in the density of radial glial staining in the cortical columns underlying the ectopia was seen. Some radial fibers extended into the space containing the ectopia and reached the overlying pia; however, most fibers stopped beneath the cell collection and seemed to be dangling. This is in contrast to those fibers at the edges of the ectopia which curved outward, as if their anchoring points had retracted as the limiting membrane space was created. Finally, a few horizontally-orientated glial fibers within the ectopia were seen.

We suggest that neurons associated with the ectopias migrate along radial glial fibers and move into a space created by an interruption in the external limiting membrane. The neurons are contained within that space by the overlying pia and the intact edges of the membrane. (Supported in part by NIH grant HD 20806.)

476.10

RADIAL GLIAL AND GFAP-IMMUNOREACTIVE FIBERS IN A REGION OF INDUCED MICRODYSGENESIS IN THE NEWBORN RAT NEOCORTEX. G.D. Rosen, D.M. Press*, G.F. Sherman, and A.M.Galaburda. Beth Israel Hospital and Harvard Med. School, Boston, MA 02215.

Freezing lesions to the left neocortex were produced in postnatal day 0 (P0) and P1 rats. The animals were sacrificed at either 1,2,3,5,7, or 10 days after the lesion by anesthetized transcardial perfusion with 4% paraformaldehyde. The brains were removed, sectioned coronally, and stained for Nissl substance with thionin, for radial glial cell-like immunoreactivity with Rat-401 antibody (obtained from S. Hockfield), and for GFAP-like immunoreactivity.

Neonatal freezing lesions produce severe cerebrocortical microdysgenesis consisting mainly of microgyri when examined in adulthood (Humphreys et al., *Soc. Neurosci Abs.*, **15**: 1120). During the first few post-lesion days, the area of damage shows an intact layer I subjacent to which there is a wide area of damage containing pycnotic cells and tissue necrosis. By P5 the typical infolding of the cortical surface is visible, and by P10 the appearance of adult-like microdysgenesis is present.

Many, but not all radial glial-like immunoreactive cells appear damaged within the first two days after the freezing lesions, showing bulbous varicosities at their foreshortened terminations lying within the area of necrosis. By P5 some radial glial-like fibers can be seen to traverse the damaged area and reach through overlying cell-dense areas up to the the external limiting membrane. From P5-P10, there is increased density of radial fibers within the area of damage, which may represent a collapse of surrounding radial fibers toward the center of the lesion.

Between P1 and P5, GFAP-like immunoreactive cells are present mostly as reactive astrocytes in the area of damage. On P7 and P10 some radial glial cells, too, express GFAP, but only within the area of neocortical damage.

These results suggest that layer I is relatively spared in this type of lesion, which may explain the paucity of molecular layer ectopic cell nests (See Sherman et al., this volume). The spared radial glial cells may permit subsequent neuronal migration to upper layers. The microgyrus may reflect physical forces of collapse. (This work was supported, in part, by NIH Grant 20806).

476.11

DEVELOPMENT OF ANTERIOR COMMISSURE ADOPTS PROGRESSIVE, NOT REGRESSIVE, STRATEGIES. R. Lent and R.Z. Guimarães*.Instituto de Biofísica, UFRJ, Rio de Janeiro 21944, Brazil.

The perinatal development of anterior commissure connections was studied in hamsters by use of dil crystals implanted either into the commissure or into the ventrolateral prosencephalon.A fascicle of growing commissural fibers was seen close to the midline on E14. On E15 it had entered the opposite hemisphere and reached the borders of the targets. No detectable waiting period was observed, since on E16 axons were already seen arborizing into most targets. All regions projecting through the commissure in adults had labelled cells on P1, namely: the anterior olfactory nuclei, olfactory tubercle , piriform cortex,nucleus of the lateral olfactory tract, bed nucleus of the stria terminalis, insular, perirhinal and entorhinal cortices and the amygdaloid complex. No evidence of topographical exuberance was ever seen. Counts of labelled neurons showed that the number of commissural cells increased gradually after birth. It was concluded that the anterior commissure adopts progressive developmental strategies, lacking the regressive phenomena that are characteristic of the more recent corpus callosum.

476.12

MYELINATION OF CORTICOHIPPOCAMPAL RELAYS CONTINUES THROUGH ADVANCED ADULTHOOD IN HUMAN BRAIN. F.M. Benes, M. Turtle, P. Farol, J.P. SanGiovanni. Quantitative and Molecular Neuroanatomy Lab, McLean Hospital, Belmont, MA 02178 and the Program in Neuroscience, Harvard Medical School.

A recent study has suggested that myelination of the perforant path (PP) and cingulum bundle (CB) in human brain is actively occurring between 11 and 17 years of age. To explore this question further, a cohort consisting of 146 post-mortem brain specimens from Boston Childrens Hospital (aged 0-33 yrs) and 21 similarly processed cases from the Massachusetts General Hospital (aged 36-76 yrs) were obtained. Transverse sections that included the hippocampal formation and entorhinal cortex had been taken at approximately the same posterior level. The extent of myelination along the superficial portion of the subiculum, presubiculum and entorhinal cortices was blindly assessed using a subjective rating scale and a computer-assisted digitization of the area occupied by myelin-staining. There was a fourfold increase in the amount of myelin-staining between 0 and 40-50 years of age that best fit a second order polynomial function with r = 0.71 and 0.76 for the subjective and areal determinations, respectively. Microdensitometric determinations of the intensity of myelin-staining showed a 31% increase up to approximately 62 years of age. These results are consistent with the idea that the numbers of myelinated axons in the PP and CB are increasing in a curvilinear fashion for approximately 5 decades, while the amount of myelin occurring in relation to individual axons may show a slower increase into the seventh decade. It will be important to know whether these developmental changes in key corticolimbic relays are genetically and/or experientially determined. Supported by MH00423 and MH42261.

476.13

NEONATAL CORTICAL AND THALAMIC LESIONS DISRUPT THE NORMAL PATTERN OF PROJECTION NEURONS, BUT NOT PRIMARY AFFERENT AXONS IN RAT TRIGEMINAL NUCLEUS PRINCIPALIS. R.W. Rhoades, C.A. Bennett-Clarke and N.L. Chiaia. Dept. of Anatomy, Medical College of Ohio, Toledo, OH 43699.

Erzurumlu and Ebner (Dev. Brain Res.; 44:302, 1988) recently showed that perinatal cortical lesions disrupt the vibrissa-related pattern of cytochrome oxidase (CO) activity in trigeminal (V) nucleus principalis (PrV), but not in V nucleus interpolaris (SpI). If normal and altered patterns of CO in PrV reflect distributions of primary afferents, then corticofugal axons must somehow affect primary afferent terminations. We tested this directly by making either cortical or thalamic lesions on P-0 and labelling V primary afferents with HRP on P-5. In other lesioned animals, we assayed the distribution of V-thalamic projection neurons via retrograde transport of fluorescent tracers. Sections from all animals were also reacted for CO. Both cortical and thalamic lesions disrupted the normal CO pattern in PrV, but not in SpI. However, the distributions of V primary afferents in both nuclei were generally normal. Cortical and thalamic damage disrupted the pattern of V-thalamic projection neurons in PrV. These results indicate that cortical and thalamic lesions alter the CO pattern in PrV via their effects upon brainstem neurons rather than primary afferents. The lack of effect in SpI probably reflects the fact that only a minority of its cells project to thalamus. Supported by DE 07734 and BNS 85 17537

476.14

THE ARACHNOID AND SUBARACHNOID SPACE IN THE HY-3/HY-3 MOUSE WITH HYDROCEPHALUS. T. Inagaki*, W. Goossens*, S. Nakahara*, D.G. McLone and P.A. Knepper. Division of Neurosurgery, Children's Memorial Hospital and Northwestern University Medical School, Chicago, IL 60614.

One mechanism for hydrocephalus may be an alteration in the development of the arachnoid and subarachnoid space (SAS). We studied the cerebrospinal fluid (CSF) outflow pathway of postnatal day 14 Hy-3/Hy-3 mice with hydrocephalus, normal littermates, and C-57 BL/6J mice by light and scanning electron microscopy (SEM).

The normal arachnoid consisted of two components: An outer continuous layer of cells adjacent to the dura and inner arachnoid trabeculae (AT) of the SAS. By SEM, normal AT consisted of highly branched cells with smooth surfaces and numerous openings and perforations: Stomata within AT as intratrabecular spaces and oval perforations between AT cells. These findings were observed in the AT of the basal cistern, the interhemispheric fissure and over the convexities. In contrast, the arachnoid of Hy-3/Hy-3 was an abnormal continuous sheet of flattened squamous cells without stomata or oval perforations over the convexities but was more typical in the interhemispheric fissure and basal cistern. These results suggest that portions of the SAS may be blocked in Hy-3/Hy-3 which may be a causative factor in hydrocephalus or may be a secondary "compressive" result of increased CSF pressure.

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476.15

EFFECTS OF PRENATAL COCAINE EXPOSURE ON BRAIN METABOLISM IN THE 21 DAY OLD RAT. D.L. Dow-Edwards, L.A. Freed, L.M. Donohue*, and H.E. Hughes. Laboratory of Cerebral Metabolism, Department of Neurosurgery, SUNY-Health Science Center, Brooklyn, N. Y. 11203.

Human infants prenatally exposed to cocaine exhibit growth and neurobehavioral deficits. Recent animal studies suggest that cocaine directly affects brain development. We have observed long-term changes in functional activity in several brain regions in the adult rat following prenatal (gestation days 8-22) and postnatal (days 1-10 or 11-20) cocaine exposure. The present study investigated rates of glucose utilization in various brain structures of weanling rats prenatally exposed to cocaine in comparison with control offspring.

Sprague-Dawley rats were gastrically intubated with 30 or 60 mg/kg/day cocaine HCl or vehicle only during gestational days 8-22. Vehicle treated rats were pair-fed/watered to rats receiving the higher dose of cocaine. A non-treated control group was also maintained. At parturition, litters from all four groups were surrogate fostered. Local rates of cerebral glucose utilization were determined in 21 day old male and female offspring using the deoxyglucose method of Sokoloff et al. (J. Neurochem. 28:987, 1977). The effects of prenatal cocaine exposure on patterns of brain metabolic activity as well as physiologic variables such as blood pressure, hematocrit and body temperature in 21 day old rat pups will be discussed.

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476.16

ETHANOL EFFECTS ON THE DEVELOPING FROG OPTIC TECTUM. L.C. Towns, P.S. Sexton* and N.J. Uray. Kirksville College of Osteopathic Medicine, Kirksville, MO 63501

Experiments were conducted to assess the effect of ethanol on morphological development of the optic tectum in bullfrog tadpoles. Groups of tadpoles were placed in a 1% (v/v) ethanol at two week intervals from the time of hatching to 22 weeks of age and all tadpoles were sacrificed at 6 months of age. In treated animals, a large continuous space develops between the ependymal and subependymal layers. When treatment is begun later, at 20 or 22 weeks, the disruption at the ependymal-subependymal boundary is generally restricted to the areas of the posterior tectum which are currently developing. Previously developed portions of anterior tectum show less disruption. Similar spaces are present in untreated controls but they do not reach the size or continuity seen in ethanol-treated animals. These data indicate that ethanol treatment induces morphological alterations in proliferative areas of the optic tectum. This disruption may result from edema due to alterations in microvasculature as previously reported in cerebellum. Supported by NIAA 07537.

476.17

ETHANOL-INDUCED ALTERATIONS IN THE MICROVASCULATURE OF THE TADPOLE BRAIN. N.J. Uray and P.S. Sexton*. Department of Anatomy, Kirksville College of Osteopathic Medicine, Kirksville MO 63501.

The purpose of this study was to examine the effect of chronic ethanol exposure on the microvasculature of tadpole brains for morphological alterations which may be associated with edema formation. Bullfrog tadpoles were raised in 1% (v/v) ethanol after which the animals were killed and the brains were prepared for light microscopy. Observations were made of the choroid plexus vasculature, along with examination of the brain tissue for signs of extravasation. Our observations suggest that after chronic ethanol exposure 1) the vessels of the choroid plexus were more tortuous in the ethanol-treated animals than in control animals and 2) the average vessel diameter in ethanol-treated animals (9.9±0.6 μm) was significantly ($P \leq 0.001$) greater than in control animals (6.0±0.2 μm). Furthermore, in the brain tissue of some of the ethanol-treated animals, red blood cells were observed in the extracellular spaces. These findings support the notion that some of the morphological changes induced by chronic ethanol exposure observed in the developing tadpole brain may be attributable to changes in the microvasculature, rather than altered neurogenesis. (Supported by NIAA grant 07537.)

476.18

AN AUTORADIOGRAPHIC STUDY OF THE EFFECTS OF REM-SLEEP ON OCULAR DOMINANCE PATCH FORMATION IN VISUALLY DEPRIVED KITTENS. J.P. Shaffery, G.A. Marks, S.G. Speciale, and H.P. Roffwarg, Dept., of Psychiatry U.T. Southwestern Med. Sch., Dallas, Tx 75235.

The influence of stimulus generated neural activity on brain development has been most convincingly demonstrated in visual system studies, where it has been shown that during the "critical period" monocular occlusion affects lateral geniculate nucleus cell sizes and the formation of ocular dominance patches (ODPs) in layer IVc of visual cortex. Monocular deprivation (MD) during the critical period results in larger than normal ODPs for the open eye and smaller patches for the occluded (deprived) eye. Previous MD studies, however, have not accounted for the possibility of an lessening of the effects of visual restriction on ODP formation that might occur during sleep. Rapid eye movement (REM) sleep is partly characterized by large amounts of endogenously generated bilateral activity that impinges upon visual thalamus and the primary visual cortical receiving area during this phase of sleep. Recent studies have indicated that combining MD with REM sleep deprivation increases the interlaminar cell size disparity usually observed in the lateral geniculate nucleus following MD alone. These data suggest the possibility of similar parallel effects occurring in the development of ODPs. The data also implicate an active role for REM-sleep in neural ontogeny and development. The data presented here test our ontogenetic hypothesis on REM-sleep and brain development. A tritiated protein was injected into either the oculed or non-occluded eyes of REM-sleep deprived or control kittens at the start of the peak of their critical period. Results from computer-aided densiotometry of ODP autoradiographs in MD kittens indicate patch size differences across groups.

DIFFERENTIATION, MORPHOGENESIS AND DEVELOPMENT: POSITION AND FORM

477.1

HOMEOBOX GENES EXPRESSED IN ADULT MOUSE CEREBELLUM. Robert F. Bulleit and Timothy A. Bunting*. Dept. of Pharmacology, University of Maryland School of Medicine, Baltimore, MD 21201

Homeobox genes encode one class of transcriptional regulatory proteins which may play a role in differentiation of brain neurons. Homeobox genes are expressed in several populations of vertebrate neurons. In invertebrates the expression of some homeobox genes is required for differentiation of certain neurons. Our interest is to identify homeobox genes that are involved in the differentiation of cerebellar neurons. We have focused on two classes of homeodomain proteins. One class includes the murine Hox family of proteins which contains a highly conserved homeodomain. The second class includes the POU/Homeo domain proteins which also contains an additional highly conserved POU domain. The polymerase chain reaction (PCR) has been used to amplify and clone cDNAs encoding members of these homeodomain protein families that are expressed in the adult mouse cerebellum. Two sets of full codon degenerate oligonucleotide primers were synthesized for use in the PCR experiments. The first set of primers is based on two regions in the homeodomain of the murine Hox proteins. The second set of primers is based on two conserved regions in the POU/Homeo domain proteins, one in the homeodomain and the second in the POU domain. The length of sequence between the two primers provides an indication of the size of DNA fragment expected to be amplified by PCR. The template DNA used for PCR was cDNA synthesized from poly A^+ RNA obtained from adult mouse cerebella. Using these two sets of primers we have been able to amplify DNA fragments of the appropriate size as observed on ethidium bromide stained 2% agarose gels. The amplified DNA was extracted from the gel and cloned into the pGEM7Z vector. Individual clones were selected and are being sequenced to determine the number of different homeodomain sequences identified from the adult cerebellum. *In situ* hybridization on tissue sections of the cerebellum will be employed to characterize the cellular distribution of the expressed homeobox genes.

477.2

IDENTIFICATION OF HBH-1, A HOMEODOMAIN-CONTAINING TRANSCRIPT FROM HUMAN FETAL BRAIN. D. J. Selski, M. Moon*, A. B. Wadhams*, P. D. Coleman, N. Thomas*, K. E. Rogers, Departments of Neurobiology and Anatomy and Dermatology, University of Rochester Medical Center, Rochester, N.Y. 14642, USA

Homeobox proteins are thought to be involved in the differentiation and function of specialized cells. In order to determine which homeobox proteins are active during neuronal development we have designed a mixed oligonucleotide probe corresponding to amino acid positions 44-55 of the conserved antennapedia homeodomain and used that probe to screen a human fetal brain cDNA expression library. Approximately 1×10^5 clones were screened using low stringency conditions and a single strongly hybidizing clone was isolated. The length of this clone, HBH-1 (human brain homeoprotein), was 1950 bases and contained an open reading frame of 400 amino acids. The full length message was judged to be 2800 base pairs by Northern blot analysis. HBH-1 sequence has been compared to known homeobox-containing sequences in the GenBank/EMBL data base and has been shown to be a unique, previously undescribed, transcript containing a region homologous to other homeodomains. Preliminary studies have demonstrated HBH-1 transcripts present in human fetal brain tissue from 11 through 21 weeks of age.(Supported by NIH grant AG 00107 and ADRDA PRG 89-120)

477.3

ALTERNATE EXPRESSION OF THE HNK-1 EPITOPE AND DEVELOPMENT OF NEURONS IN RHOMBOMERES OF THE CHICK EMBRYO. S.C. Kuratani. Department of Anatomy, Medical College of Georgia, Augusta, GA 30912-2000.

To investigate developmental changes occurring in central nervous system and neural crest, a series of chick embryos were immunostained with several monoclonal antibodies. HNK-1-immunoreactivity appeared in rhombomeres(r)3 and r5 around stage 12. This immunoreactivity was the most intensive at stage 15, when r2 and r4 were not stained. This alternating pattern is similar to the Krox-20 gene expression in the mouse embryo (Wilkinson et al., 1989a). At levels of r2 and r4, neural crest cells were attached to the hindbrain. An accumulation of neurons was observed in these rhombomeres near this attachment. The above observations seem to suggest that the alternate HNK-1 immunoreactivity in rhombomeres might be related to pair-rule-like segmentation of the nervous system in concordance with the branchiomerism. This phenomenon is the first example of a phenotypic differentiation of CNS seemingly related to homeobox-containing gene expression.

477.4

POSSIBLE ROLE OF BASIC FIBROBLAST GROWTH FACTOR IN THE DEVELOPING AND REGENERATING RETINA OF GOLDFISH. Pamela A. Raymond and Linda K. Barthel, Univ. Michigan, Dept. Anatomy & Cell Biology, Ann Arbor, MI 48109

Antibodies to basic FGF isolated from bovine brain bind to various specific cells or laminae in the larval and adult goldfish retina; the most intense immunoreactivity (blood vessels excepted) is in horizontal cells (HCs) and outer plexiform layer (OPL). This observation is intriguing since in this retina, rod photoreceptors are added continuously through mitotic divisions of rod precursor cells located along the edge of the outer nuclear layer adjacent to the OPL. Double label studies with GABA antibodies (which stain the predominant type of HC in fish) suggest that some but not all of the FGF immunoreactivity co-localizes with GABAergic HCs. It has been proposed that GABAergic HC's may play an organizing role during development of photoreceptors and outer retina. We have tested the hypothesis that FGF may be involved in this function by inserting ethylene vinyl acetate pellets containing FGF antibodies into the eyes of normal fish and those with regenerating retinas. Preliminary results indicate that the cytoarchitecture of developing retina may be affected by FGF antibodies: laminar organization is disrupted and some neurons are in ectopic positions. Supported by NIH EY04318.

477.5

NUCLEATION OF STRIATAL PATCHES: EARLY BORN PATCH COMPARTMENT NEURONS SHOW GREATER ADHESIVENESS THAN LATER BORN PATCH COMPARTMENT NEURONS. J.G. Johnston, L.A. Krushel and D. van der Kooy, Neurobiology Research Group, Department of Anatomy, University of Toronto, Toronto, Ontario, Canada, M5S 1A8.

The mammalian striatum can be divided into the patch and matrix compartments based on the distributions of neurochemical markers and anatomical connections and based on striatal development. The patch and matrix compartment neurons are born at different times during rat development, with majority of patch neurons becoming postmitotic (embryonic (E) days 13-15) before the majority of matrix neurons (E18-20). Cell lineage studies suggest that the striatal compartments develop from separate pools of precursor cells. The neurons of the patch compartment are adhesive to one another, as shown by their ability to selectively reaggregate in culture. In order to investigate within compartment adhesion, we asked if adhesion differed amongst neurons of the patch compartment. Maternal injections of one of ^{3}H-thymidine or bromodeoxyuridine were made on E13 and were followed in the same pregnant rats by injections of the alternate label on E15, in order to mark new postmitotic patch neurons at early and late times of patch neurogenesis. Animals sacrificed at postnatal day 30 revealed that early born neurons are preferentially located towards the centers of patches, whereas later born patch neurons are preferentially distributed towards the peripheries of the patches. Thus, in addition to a previously described qualitative difference in the self-adhesiveness of striatal patch neurons versus the lack of such a self-adhesiveness among striatal matrix neurons, there is also a difference in adhesiveness among neurons of the patch compartment. The increased adhesiveness of the earlier born patch neurons may serve to nucleate the patches during striatal development.

477.6

POSTMITOTIC STRIATAL PATCH AND MATRIX NEURONS INTERMIX BEFORE COMPARTMENTS ARE FORMED. L.A. Krushel, G. Fishell and D. van der Kooy Dept. of Anatomy, Univ. of Toronto, Toronto, Ont. Canada, M5S 1A8.

Neurons of the patch compartment in the rat striatum become postmitotic earlier in neurogenesis than neurons of the matrix compartment. The previously demonstrated selective adhesion of patch cells to one another may be an important mechanism in the development of striatal compartmentalization. We examined both *in vivo* and *in vitro* whether the selective adhesion of patch cells is expressed before or after the migration of the majority of the matrix neurons into the striatum. *In vivo*, patch neurons were labeled by a fluorescent retrograde tracer (true blue) injected into the substantia nigra on embryonic day (E) 19 (patch cells are the first neurons to project out of the striatum). Matrix neurons were labeled with a maternal injection of bromodeoxyuridine (Brdu - a birthdate marker) on E18. On E20 true blue labeled patch cells were found intermixed with the Brdu labeled matrix neurons in the medial striatum, however further laterally small clusters of labeled patch cells were present. By postnatal day 2 there was a complete segregation of the clusters of labeled patch neurons from the matrix neurons in the striatum. This process was also modeled *in vitro*. The patch and matrix compartments were labeled *in vivo* with different birthdate markers (^{3}H-thymidine or Brdu) on E13 and E18, respectively. On E20 the striatal tissue was removed, dissociated and reaggregated in suspension cultures. After 1 day *in vitro*, labeled patch and matrix cells were randomly intermixed within the reaggregates. Examination of the cultures after 2.5 and 4 days *in vitro* revealed clumping of the labeled patch cells towards the centers of the reaggregates. Over this same period, the labeled matrix neurons did not clump and were dispersed towards the periphery of the reaggregates. Thus, the selective adhesiveness of patch neurons is either expressed after the later born matrix neurons begin to migrate into the striatum or is unable to overcome the force of the massive migration of matrix neurons. We conclude there is a migratory phase when committed patch and matrix neurons intermix before expressing their adhesive phenotype in the postmitotic striatum.

TRANSPLANTATION: RECEPTOR EXPRESSION

478.1

RAT FETAL NEOCORTEX TRANSPLANTS HAVE BOMBESIN/GRP RECEPTORS. J.M. Rosenstein, R. Getz* and T.W. Moody, Depts. Anatomy and Biochemistry & Molecular Biology, George Washington Univ. Sch. Med., Washington, D.C. 20037.

Fetal brain grafts contain numerous neurotransmitters and receptors. Of particular interest is bombesin/gastrin releasing peptide (BN/GRP) whose receptors develop on fetal neocortex transplants into the adult fourth ventricle (Getz et al., Neurosci. Lett. 79:97 (1987). BN/GRP functions as a potent satiety and grooming agent in the rat CNS and stimulates the growth of certain neuroendocrine cells. Here the binding properties of these fetal cortex transplants were investigated using in vitro autoradiographic techniques. High densities of (^{125}I-Tyr^4)BN grains developed in fetal cortex transplants into the host ventricle or adult cortex but not on cerebellar or superior cervical ganglia transplants. These data indicate that the development of BN/GRP receptors is a function of the transplant and not the host tissue. The autoradiographic grains, which were analyzed on an Amersham RAS 3000 densitometer, developed 3 weeks after tranplantation in the graft tissue and were retained for 3 months. The amount bound was a function of (^{125}I-Tyr^4)BN concentration. Scatchard analysis indicated that radiolabeled (Tyr^4)BN bound with high affinity (Kd = 4 nM) to a single class of sites (Bmax = 110 fmol/mg protein). GRP and GRP$^{18\text{-}27}$ but not GRP$^{1\text{-}16}$ inhibited specific (^{125}I-Tyr^4)BN binding with high affinity (IC_{50} = 2, 5 and >10,000 nM respectively). Also, BN receptor antagonists such as (Psi^{13,14}, Leu^{14})BN and (D-Arg^1, D-Pro^2, D-Trp^{7,9}, Leu^{11})substance P competed for the (Tyr^4)BN binding sites (IC_{50} = 100 and 1000 nM respectively). Because BN/GRP receptors develop on fetal cortex transplants, they may facilitate the growth of the graft tissue. Supported by NIH grant NS-17468 and NSF grant 88-15133.

478.2

CHANGES IN STRIATAL DOPAMINE RECEPTOR BINDING FOLLOWING ADRENAL MEDULLA GRAFTS. E.J. Curran and J.B. Becker. Neuroscience Program and Department of Psychology, The University of Michigan, Ann Arbor, MI 48109.

We have previously shown that intraventricular adrenal medulla (AM) grafts transplanted adjacent to the dopamine (DA) denervated striatum produce a decrease in rotational behavior induced by either amphetamine (AMPH) or apomorphine (APO). Experiments using quantitative autoradiography were conducted to determine whether changes in striatal DA receptors are associated with this behavioral recovery.

Adult rats with unilateral substantia nigra lesions were tested for APO- and AMPH- induced rotational behavior prior to and after receiving intraventricular AM grafts. Ten to twenty days following the last drug treatment, the rats were decapitated, the brains were rapidly removed, frozen on dry ice, and stored at -70°C. Twenty micron coronal sections through the striatum of control animals and animals with behaviorally effective or ineffective AM grafts were assayed for [^{3}H]SCH 23390 binding to D1 receptors or [^{3}H]spiperone binding to D2 receptors. Preliminary experiments revealed that D2 receptor binding increased in the DA denervated striatum. Behaviorally effective AM grafts attenuated this increase. In contrast, no apparent change was seen in D1 receptor binding in the DA denervated animals. (Supported by grants NS 22157 & UM 363669).

478.3

BEHAVIORAL RECOVERY ASSESSED WITH D_1 AND D_2 DOPAMINE AGONISTS AFTER VENTRAL MESENCEPHALIC GRAFTS INTO DOPAMINE DENERVATED RAT STRIATUM.

J.B. Becker[1], M.A. Ariano[2], & D.K. McFarlane[1]. [1]Psychology Dept & Neuroscience Prgm, Univ. Michigan, Ann Arbor, MI 48104; [2]Anatomy and Neurobiology, UVM, College of Medicine, Burlington, VT 05405.

Stimulation of both D_1 and D_2 dopamine (DA) receptor subtypes synergistically activates behavior mediated by the intact striatum. Following unilateral DA denervation, this facilitatory receptor interaction is thought to be uncoupled; administration of either a D_1 or a D_2 DA receptor agonist will induce rotational behavior (**J. Pharm. Exp. Ther.**, 247:180, 1988). Previous experiments have demonstrated that turning behavior induced by either apomorphine (APO) or amphetamine (AMPH) is decreased after grafts of fetal dopaminergic tissue into the DA denervated striatum. Since APO and AMPH will activate both DA receptor subtypes, this experiment was conducted to determine whether behavioral recovery is associated with a single DA receptor population.

Female rats with a unilateral 6-OHDA lesion of the substantia nigra underwent behavioral testing before and 2-3 months after intrastriatal grafts of fetal ventral mesencephalon. Animals were tested with: the full D_1 agonist, SKF82958 (0.5 mg/kg); the D_2 agonist, LY171555 (0.1 mg/kg); and AMPH (5.0 mg/kg). Survival of DA containing grafted cells was confirmed histologically 4 months post-graft with catecholamine histofluorescence.

Preliminary data indicate that the transplantation of DA containing fetal brain tissue can produce decreases in rotational behavior induced by SKF82958 (range = -18 to -75%), LY171555 (-15 to -99%) and AMPH (-15 to -96%). We conclude that behavioral recovery induced by fetal mesencephalic grafts in this animal model of Parkinson's Disease involves compensatory responses by both D_1 and D_2 DA receptor subtypes.

[Supported by USPHS NS 22157 (JBB) and NS23079 (MAA)].

478.4

D_1 AND D_2 RECEPTOR MORPHOLOGY AFTER PLACEMENT OF VENTRAL MESENCEPHALIC GRAFTS IN DOPAMINE DEPLETED RAT STRIATUM.

M.A. Ariano, J.B. Becker,[1] L.D. McVittie,[2] & D.R. Sibley[2]. Anatomy & Neurobiology, UVM College of Medicine, Burlington, VT 05405; [1]Psychology Dept., Univ. Michigan, Ann Arbor, MI 48104; & [2]ETB, NINDS-NIH, Bethesda, MD 20892.

There are two distinct dopamine (DA) receptor subtypes. The D_1 receptor stimulates adenylyl cyclase. Striatal D_1 binding sites are found morphologically associated with neurons containing cAMP immunoreactivity. Stimulation of striatal D_2 receptors inhibits cAMP production. Striatal DA denervation produces specific receptor changes; the association between D_1 binding sites and cAMP immunoreactivity is abolished and there is an increase in the number of D_2 receptors. This study was initiated to determine whether transplanting fetal mesencephalon into the DA-denervated striatum could induce recovery of these morphochemical indices of DA receptor function.

Female rats with a unilateral 6-OHDA nigral lesion, underwent behavioral testing (results reported separately) before, and 2-3 months after transplantation. Cellular distributions of the DA receptors were examined after 4 months. The striatal distribution of both DA receptor subtypes was determined by application of rhodamine derivatized antagonist ligands (**PNAS 86**:8570, 1989). The morphochemical aggregation pattern of D_1 receptors was assessed by *in vitro* autoradiography with immunohistochemical staining of cAMP (**Brain Res. 443**:204, 1988). D_2 receptor distribution was further characterized by specific antibodies generated against portions of the peptide sequence for the native protein (**FASEB J. 4**:A601, 1990). Preliminary data suggests that the transplantation of DA-containing fetal brain tissue provides a sufficient milieu for recovery of the DA receptor distribution. The data suggest a morphological basis for behavioral improvements induced by grafts of fetal ventral mesencephalon in this animal model of Parkinson's Disease.

[Supported by USPHS NS 23079 (MAA) and NS 22157 (JBB)].

478.5

AUTORADIOGRAPHIC STUDY OF D-1 AND D-2 RECEPTORS IN THE EFFERENT REGIONS OF THE STRIATUM AFTER CHRONIC TREATMENT WITH L-DOPA IN 6-OHDA LESIONED RATS WITH OR WITHOUT NIGRAL GRAFT. L. Rioux, D. Gaudin, P.J. Bédard, C. Gagnon and T. DiPaolo. Lab Neurobiol. Dept. Anatomy and Dept Mol. Endocrinol.and School of Pharmacy Univ. Laval, Québec, (Qc), CANADA G1K 7P4.

We have previously shown that fetal DA neuron transplants in the rat striatum prevent supersensitivity of the DA receptors induced by denervation itself or by repeated administration of L-Dopa (Gaudin, et al., Soc. for Neurosc.abstr.,15: 1355,1989). In the present work, we have studied the effects of a similar striatal graft on dopamine D-1 and D-2 receptors in the target structures of the striatum. Two group of rats were prepared with a unilateral lesion of the nigro-striatal DA pathway with 6-OHDA. One month later, one group received a graft of 1.5 $X10^6$ midbrain cells. Each group of animals received 14 injections of L-Dopa. After four months the animals were sacrified for autoradiography of the D-1([^{3}H]-SCH 23390) and D-2 ([^{3}H]-Spiperone) receptors. The results show a decreased density of D-1 receptors in the GP (77%), EP (36%) and no change in the SN of the grafted side compared to the lesioned ungrafted side of the second group. On the other hand, an increased density of D-2 receptors was observed in the GP of the grafted side (25%) and surprisingly of intact side of grafted animals (77%). In the SN, the density of D-2 dopaminergic receptors was decreased on the lesioned and grafted side (57%). Our results show that a fetal nigral graft can modulate the changes of D-1 and D-2 receptors caused by dopaminergic denervation and by chronic levodopa treatment in striatal target structures. The modifications of dopaminergics receptors in the efferents regions of striatum of grafted animals may also explain the absence behavioral superpersensitivity (Gaudin et al., Br. Res.,506: 1, 166-168, 1990) after chronic treatment with L-Dopa.

Supported by MRC of CANADA

478.6

EFFECT OF EMBRYONIC DOPAMINERGIC NEURONS TRANSPLANTED TO THE 6-OHDA DENERVATED RAT ON DOPAMINE D_1 AND D_2 RECEPTOR DENSITIES : AN AUTORADIOGRAPHIC STUDY.

F. MENNICKEN, D.N. ABROUS*[1], J.P. HERMAN*[1], M. LE MOAL[1], C. FEUERSTEIN and M. SAVASTA, INSERM-LAPSEN U.318, Pavillon de Neurologie, CHU de Grenoble, 38043 Grenoble cedex and 1. INSERM U.259, Domaine de Carreire, 33077 Bordeaux cedex.

Three experimental groups (control group, lesion group and lesion + graft group) of rats were used for this study. The mesotelencephalic system of adult animals was unilaterally destroyed by 6-OHDA injected into the medial forebrain bundle. Three weeks after the lesion, a cell suspension containing embryonic dopaminergic neurons was implanted into the denervated striatum. Six months after, animals were sacrified. D_1 and D_2 dopamine (DA) receptors densities were examined autoradiographically using ^{3}H-SCH 23390 and ^{3}H-Spiperone (or ^{3}H-Raclopride) respectively. The distinction between reinnervated and non reinnervated striatal regions by the graft was achieved by the autoradiographic visualization of a DA uptake inhibitor, the ^{3}H-GBR 12935, on adjacent sections. Autoradiograms were quantified by image analysis (SAMBA 2005). The results obtained can be resumed as follows:

- Six months after the DA denervation, the density of D_1 receptors was not modified in the denervated striatum, confirming previous results obtained after a post lesional delay of 1 month (SAVASTA et al, Biogenic Amines 4-6 : 419, 1987). On the other hand, the density of D_2 receptors was increased (25 to 45%) preferentially in the lateral part of the striatum as previously described (SAVASTA et al, Neurosci. Lett., 74 : 180, 1987).
- After implantation of embryonic DA neurons in the depleted DA striatum, the D_2 DA receptor density returned to control values in the reinnervated striatum.

These results provide evidence that functionnal recovery of embryonic DA neurons graft is probably associated to a receptor mediated mechanism.

478.7

EFFECT OF EMBRYONIC DOPAMINERGIC NEURONS TRANSPLANTED TO THE 6-OHDA DENERVATED RAT ON THE EXPRESSION OF THE mRNA CODING FOR D_2 RECEPTOR : AN IN SITU HYBRIDIZATION HISTOCHEMISTRY STUDY.

M. SAVASTA, M. CHRITIN, D.N. ABROUS*[1], J.P. HERMAN*[1], M. LE MOAL[1] and C. FEUERSTEIN, INSERM-LAPSEN U.318, Pavillon de Neurologie, CHU de Grenoble, 38043 Grenoble cedex and 1. INSERM U.259, Domaine de Carreire, 33077 Bordeaux cedex.

Three experimental groups (control group, lesion group and lesion + graft group) of rat were used. The mesotelencephalic system of the animals was unilaterally destroyed by 6-OHDA injected into the medial forebrain bundle. Three weeks after the lesion, a cell suspension containing embryonic dopaminergic neurons was implanted into the denervated striatum. Six months after the graft animals were sacrified. Autoradiographic studies carried out in parallel from consecutive sections of the same animals evidenced an increase of D_2 dopamine (DA) receptors density which was reversed by the implantation of embryonic dopaminergic neurons. In the present study, using in situ hybridization techniques, we examined whether levels of mRNA coding for D_2 DA receptors vary in the same way than those of the D_2 receptor protein itself. [^{32}P]-labelled oligonucleotide derived from the coding region of the rat DA D_2 receptor cDNA has been used as a probe to localize in the rat brain sections the cells containing the mRNA coding for this receptor. The distribution of mRNA was comparable to that of the dopamine D_2 receptor binding sites as visualized in adjacent sections by autoradiography of ^{3}H-Spiperone or ^{3}H-Raclopride. The lateral part of the striatum presented high mRNA content comparatively to the other subregions of this structure. In the denervated striatum, the mRNA levels were preferentially increased in the lateral striatum as described for the supersensitive D_2 receptors, after implantation of embryonic dopamine neurons the expression of mRNA for this receptor was normalized. These results provide evidence that functional recovery of DA neurons graft is mediated at least in part through the modulation of the genetic expression of the D_2 receptor.

479.1

WEAK ELECTRICAL FIELDS SPEED UP REGENERATION OF MOTONEURONS IN THE CRUSHED SCIATIC NERVE OF NINE MONTH OLD RATS. J.I. Campbell and B. Pomeranz. Dept. of Zoology, Univ. of Toronto, Toronto, Canada M5S 1A1

Our lab has previously reported that motoneuron regeneration is accelerated when treated with weak electrical fields in cut and sutured but not crushed sciatic nerve of 10 week male rats (McDevitt, L. et al., Brain Res., 416:308, 1987). The present study was conducted to determine whether: 1. the speed of nerve regeneration is slowed with age, and 2. if so, will 10 μA direct current stimulation accelerate regeneration in the old animals with crushed nerves?

Seventeen 10 week male Wistar rats underwent a crush of the right sciatic nerve. This group was called Young Crush (YC). In 18 rats the crush was made at 9 mo of age (Old Crush =OC). Seventeen 9 mo rats received a crush and had battery driven wick electrode devices implanted in the neck region with the cathode distal wick passing by the site of the lesion (Old Crush Cathode Distal = OCCD). These implants drove a current of 10 μA by the nerve throughout the recovery period. Fifteen 9 mo rats underwent a crush and were implanted with sham devices (Old Crush Sham = OCS).

On post-operative day 29, EMG's were recorded by stimulating the sciatic nerve transcutaneously via micro-alligator clips while recording pins were inserted in one of 12 identified sites in the rat's right foot. 70.8% of all sites were innervated in the YC while only 26.7% of the sites were innervated in the OC suggesting that regenerative speed slows with age. Also, the OCCD had 68.8% of all sites innervated, while the OCS had only 18.8%, indicating that DC fields speed up the rate of regeneration.

479.2

QUESTIONING THE PROPOSED ENHANCEMENT OF PERIPHERAL NERVE REGENERATION BY APPLIED ELECTRIC FIELDS. M. E. McGinnis. Center for Paralysis Research, School of Veterinary Medicine, Purdue University, West Lafayette, IN 47907.

Several papers have been published reporting an enhancement of the rate of peripheral nerve regeneration in rats due to the application of weak DC electric fields. Having been unable to demonstrate any effect of such fields on peripheral nerve regeneration in guinea pigs, I undertook to repeat as closely as possible two of the published positive reports. An experiment by Politis, Zanakis, and Albala (J. of Trauma **28**:1375-1381, 1988) consisted of placing a transection and anastomosis site within a silicone tube with electrodes at either end. An unregulated current source passed 1.5 μA through the tube for 12 days with a resulting 4 fold increase over controls in the number of neurofilament positive profiles found 14 mm distal to the anastomotic site. My repeat of this experiment showed that the devices only pass 0.23 μA, most of which follows the low resistance pathway around the outside of the tube rather than going through it. At the time and distance indicated, I found no difference in the number of neurofilament positive profiles, or myelinated or unmyelinated fiber density.

Another paper (Román *et al.* Exp. Neurology **98**:222-232, 1987) reported a 5.5 fold increase in the number of myelinated fibers regenerating through a silicone tube (across a 5mm gap) when a cathode delivering 10 μA was located in the center of the tube. A repeat of this experiment with a larger sample size than originally used demonstrated no effect of the applied fields.

From these experiments it is apparent that the effects of applied fields on peripheral nerve regeneration is still open to question, and should remain so until a robust, repeatable effect can be demonstrated by several labs. Supported by NIH grant NS 26251

479.3

EFFECT OF VARIOUS ELECTRICAL PARAMETERS ON PERIPHERAL NERVE REGENERATION. M.F. Zanakis*, L. Nguyen, C. Kaiser, A. Feller, S. Fettahlioglu, and B. Hallas. *American Bio-Interface Corporation. New York, NY and New York College of Osteopathic Medicine, Dept. of Neuroscience, Old Westbury, NY 11568.

Studies have shown that cathodally-directed D.C. stimulation of damaged mammalian peripheral nerves results in an increased rate of regeneration toward target muscles. Constant D.C. current was regulated by a resistor or an I.C. at two voltage levels in the lesioned PNS. Rat sciatic nerves were transected and then frozen with dry ice, and a galvanic nerve cuff was placed over the lesion site with the cathode located 10 mm distal to the lesion. The electrodes delivered A) either 1.4 or 14uA at 1.4V limited by a resistor or B) delivered 1.4 or 14uA at 9V limited by an I.C. Animals were allowed to survive for 6 to 15 days post lesion/treatment. Frozen serial sections of the nerve were reacted with fluorescent antibody to neurofilament protein. Results at 8 days showed that the electrodes at 1.4 and 14uA (at 1.4V) whose current was limited by a resistor demonstrated equivalent numbers of axons, yet contained significantly more axons than the I.C. regulated group animals, whose axon counts were also equivalent in that group. At 15 days, the axon counts were elevated by about 20% in all groups, but no statistical differences were found between groups.

479.4

REGENERATION OF DORSAL ROOT AXONS IS INFLUENCED BY INTERCELLULAR REACTIONS IN THE DORSAL ROOT GANGLION. X. Lu* and P.M. Richardson. Montreal General Hospital and McGill University, Montreal, Canada H3G 1A4.

Do cellular interactions within the dorsal root ganglion contribute to the regenerative propensity induced in sensory neurons by peripheral nerve injury? To study this question, axonal regeneration was assayed in rat L_5 dorsal spinal roots following a variety of interventions designed to inhibit or enhance such regeneration. Counts of thinly myelinated fibres 17 days after crush injury were increased by injection into the ganglion of isogenous macrophages or the inflammatory agent C. Parvum. Chronic intrathecal infusion of mitomycin C did not significantly retard the relatively vigorous dorsal root regeneration induced by cutting of the sciatic nerve. As assessed by thymidine radioautography, this infusion of mitomycin C did reduce the proliferation of satellite cells that normally follows sciatic nerve transection. These observations raise the possibility that perikaryal regenerative responses after nerve injury are not directly induced by axonal interruption but indirectly by secondary reactions of satellite glial cells surrounding the nerve cell body. However, satellite cell proliferation is not essential for this stimulation.

479.5

TRANSDIFFERENTIATION OF *XENOPUS* RETINAL PIGMENT EPITHELIAL CELLS INTO GLIAL-LIKE CELLS IN CULTURE. D.S Sakaguchi. Dept. Biol. B-022, UCSD, La Jolla, CA 92093

The retinal pigment epithelium (RPE) of amphibians can transdifferentiate into neurons and lens cells in culture. I report here that the RPE, in addition, is capable of transdifferentiating into glial-like cells. For these studies the posterior two-thirds of larval *Xenopus* (stages 45-53) RPE was enzymatically dissociated and placed into primary culture. After 1 week RPE cells were harvested and replated under limiting dilution conditions. Using this approach several clonal cell lines were established from single RPE cells, 2 of which are discussed here. To identify different cell types which transdifferentiated in culture a panel of cell-type specific antibodies was used. Initially all RPE cells were labeled by an RPE specific mAb (XAR1), and were not labeled by glial cell markers (anti-vimentin, anti-GFAP or R5 mAb). However, after two months in culture, RPE clonal cell lines had lost their pigmentation and were no longer immunoreactive to the RPE specific mAb. In addition, the RPE derived clonal lines were now labeled by the glial cell markers. These cell lines were then examined for their ability to promote neurite outgrowth from embryonic retinal explants. Although collagen alone was relatively ineffective at promoting neurite outgrowth, collagen substrates directly conditioned by the RPE derived cell lines were capable of promoting neurite outgrowth following chemical extraction with Triton X-100. This ability of RPE cell line derived extracellular matrices to support neurite outgrowth is a feature shared with the XR1 cell line, a *Xenopus* glial cell line derived from the retinal neuroepithelium.

479.6

ACTH PEPTIDES IMPROVE ENDPLATE PARAMETERS DURING THE REGENERATION OF THE DEVELOPING PERIPHERAL NERVOUS SYSTEM. L.A. Zuccarelli and F.L. Strand. Department of Biology and Center for Neural Science, New York University, Washington Square, New York, New York 10003.

ACTH/MSH peptides accelerate peroneal and sciatic nerve regeneration in the adult rat, improving qualitative and quantitative aspects of extensor digitorum longus (EDL) muscle reinnervation, and affecting the morphology of the neuromuscular junction (NMJ) [1,2,3,4]. These peptides act effectively during critical developmental periods [5,6]. Here we investigate the role of α-MSH and ACTH 4-10 on the regeneration of the developing motor system after trauma. Two-day old Sprague-Dawley rats are subjected to sciatic nerve crush with a #5 forceps producing a .5 mm wide lesion. Peptide treatment (10 μg/kg/48 h) is from time of surgery continuing 6 or 8 days. At days 7 and 15, the EDL muscles are fixed in situ and the NMJs are prepared for light microscopy with a modified silver-cholinesterase stain. At day 7, both peptides significantly increase interior endplate branching, while MSH also increases area and perimeter as compared to saline controls. Muscle fiber diameter is decreased with both peptides, significantly by ACTH 4-10 only. At day 15, both peptides increase NMJ area, perimeter and fiber diameter; interior branching is comparable in all groups. These results continue to support the efficacious role of ACTH/MSH peptides in PNS regeneration. 1. Strand & Kung,(1980) Peptides 1:135-138; 2. Saint-Come & Strand (1985) Peptides 6(s.1)77-83; 3. Zuccarelli & Strand (1988) Soc. Neuro. Abs. 14 #203.1;4. Biljsma et al (1983) Acta Neuro 62:24-30. 5. Rose et al.(1988) Peptides 9:151-156; 6. Frischer & Strand(1988) Exp.Neurol. 100:531-541. Supported by Biomeasure, Inc.

479.7

ENDPLATE MORPHOLOGY AND WALKING TESTS ARE ENHANCED BY BIM 22015 (ACTH 4-10 ANALOG) DURING PERIPHERAL NERVE REGENERATION. T.S. Lee, S.J. Lee, I. Bell*, K. Foster*, and F.L. Strand. Department of Biology and Center for Neural Science, New York University, Washington Square, New York, New York 10003.

Sprague-Dawley rats (175-200g) are subjected to peroneal nerve crush under 8% choral hydrate anesthesia. A #5 forceps is used, resulting in a 1 mm wide lesion. Starting at the time of the surgery, an ACTH 4-10 analog (BIM 22015, 0.1μg/48hrs i.p.) is administered for 5 or 7 days. 5 days after nerve crush BIM-22015 treated animal show a significant increase in endplate perimeter and nerve terminal interior branching. At 7 days post-surgery peptide treatment increases endplate area, internal branching and muscle fiber diameter.

The walking test consist of having the animals walk up an inclined pathway, after having their feet dipped in non-toxic ink[1]. The resulting footprints permit the measurement of toespreads, print length and the calculation of the Peroneal Function Index[2]. The contralateral foot is used as a control for the lesioned foot and parameters are compared to saline-treated controls. BIM 22015-treated rats show enhancement of recovery as determined by these parameters. Several ACTH peptide fragments (ACTH 4-10 and a ACTH 4-9 analog, Org 2766) improve peripheral nerve regeneration and endplate morphology following nerve crush[3]. This ACTH 4-10 analog, BIM 22015, can now be included in this family of neurotrophic peptide. 1. De Medinaceli, L., *et al.,* (1982) *Exp. Neurol.* 77:634; 2. Bain, J.R., *et al.,* (1989) *Plas. Recon. Sur.* 83:129-136; 3. Strand, F.L., *et al.,* (1989) *Prog. Neuro* 33:45-85. Supported by Biomeasure Inc.

479.8

SEX DIFFERENCES IN REGENERATION AFFECT ACH RECEPTOR CONCENTRATION AND MOTOR RECOVERY J. Kume and F.L Strand, New York University, Dept. Biology & Neural Science, NY, NY 10003

Sex steroids have recently been shown to affect the non-androgen sensitive muscle, the extensor digitorum longus (EDL)(Segarra,A.,Bleisch,B. and Strand,F.L. 3rd Intnl Nerve Regen. Symp., Dec.1989). We thus investigated the relationship between acetylcholine receptor (AChR) concentration and motor recovery in the EDL, after nerve-crush in castrated and normal rats. Male and female rats were divided into 3 groups: sham-crushed; nerve crushed; and castrated, nerve crushed. Under anesthesia, the peroneal nerve was crushed at the site of innervation. For the AChR assay, males were castrated using an LHRH antagonist (BIM-21009, Biomeasure,Inc., 5mg/kg,s.c.). Females were surgically castrated. AChR concentration was found by radiolabelled binding of $I^{125}\alpha$-bungarotoxin. Motor tests were done on rats used for the AChR assay, and in a separate trial where some males underwent nerve-crush and surgical castration during the peripubertal stage. Digit Distance 1-5 (DD1-5) was measured by the method described by Bain (Plastic & Reconst. Surg.,1989, 83:129). Nerve crush increases AChR concentration, especially in castrated animals. At 9 days post-crush, a sex difference between castrated groups appears, males having 28% higher AChR concentration than females. This difference is not seen in rats with normal steroid levels. However, when relating this difference to DD1-5, only denervated, non-castrated males had a larger toespread than their female counterparts. In the second trial, no such motor difference was noted for any nerve-crushed groups. In fact, when comparing relative DD1-5, print length, and peroneal functional index ratios, these males recovered less than females. Crushing during the peripubertal stage, when male testosterone levels have not yet peaked to adult levels restrains the positive neurotrophic pattern of recovery from occurring. (Supported by NIMH training grant# MH18882-03.)

479.9

THE EFFECT OF LOCAL ADMINISTRATION OF EXTRACELLULAR MATRIX AND NERVE GROWTH FACTOR ON FUNCTIONAL OUTCOME FOLLOWING NEONATAL THORACIC SPINAL CORD TRANSECTION IN THE RAT. B.B.Walters, J.R.Encarnacion* and T.E.Melin*. Div. of Neurosurgery, Univ. of North Carolina at Chapel Hill, NC 27599-7060.

40 Sprague-Dawley rats have been studied in a chronic spinal injury protocol designed to demonstrate the effects of local application of extracellular matrix with or without additional growth factors on functional outcome. An open procedure was performed under methoxyflurane anesthesia with an operating microscope in the mid-thoracic region within 36 hours of birth. 13 had laminectomies alone (LAMI), 11 had intradural transection and one segment myelectomy (TRANS), 9 had myelectomies with implantation of approx. 7 µl of matrigel (ECM), and 7 had implants containing approx. 3.5 µg of 2.5S NGF (NGF). The animals were evaluated by the combined behavioral score of Wrathall (Exp.Neurol. 88:123, 1985) 3 times weekly for 15 weeks in blinded fashion. The LAMI animals performed significantly better ($p<0.00003$, ANOVA) than all other groups on weeks 3-15. From 5-8 weeks ECM animals outperformed TRANS animals ($p<0.05$ at 5 & 8, $p<0.001$ at 6). In contrast, NGF animals performed the same as TRANS animals through week 6 and then performed more poorly ($p<0.05$ to <0.001). The neuroanatomical correlates of these results are being investigated. Supported by the American Paralysis Association.

479.10

A NEUROTROPHIC FACTOR RELEASED FROM PRIMARY HEPATOCYTE CULTURES PROMOTES NEURITE REGENERATION FROM NERVE-TRANSECTED TERMINALS OF ADULT MOUSE DORSAL ROOT GANGLIA IN VITRO. H.HORIE[1], Y. BANDO*[1], N. FUKUDA*[2], AND T. TAKENAKA, Dept. of Physiol. and [2]Surgery, Sch. of Med., Yokohama City Univ., Yokohama, 236 Japan

Dorsal root ganglia with nerve fibers, dissected from 3-month-old mice, were embedded in collagen gel. After perfusion with 0.05% collagenase, livers of 3~4-month-old mice were dissected and dissociated by mild pipetting. Hepatocytes were cultured on a collagen coated dish in a Ham's F12 medium with 10% fetal calf serum . When the explants were cultured in the hepatocyte condition medium, the number and average length of regenerating neurites were 25.8 and 459µm after 3 day in culture, 33.0 and 617 µm after 5 days and 40.0 and 1020 µm after 8 days. When the explants were cultured in a serum containing medium with NGF, the number and average length were 18.0 and 394 µm after 3 day in culture , 16.9 and 644 µm after 5 days and 12.4 and 992 µm after 8 days. Similar effects were observed in a serum free hepatocyte condition medium. The hepatocyte condition medium promotes the growth as well as NGF, but the effects on the number of regenerating neurites and the cell survival are more remarkable than those of NGF. These results indicate that hepatocytes might secret an essential factor for regeneration from nerve-transected terminals of an adult mouse.

479.11

ORGANIZATIONAL PATTERNS OF REGENERATING NERVE FIBERS AFTER SPINAL CORD COMPRESSION INJURY. C.P. Barrett, R. Rees*, and L. Guth*, Dept. of Anatomy, Sch. Med., U. MD, Balt.

Spinal lesion sites in untreated compression injury of rat spinal cords reveal necrosis that begins a few days post injury and eventually spreads rostrocaudally for 3-4 spinal segments. In such lesions neuritic outgrowth is severely restricted or absent. However, similar injury in lipopolysaccharide treated rats (0.1 mg/kg i.v. twice daily for 8 weeks) is followed by less severe reactive changes, reduced necrosis and gliosis, and the formation of cellular bridges between the cranial and caudal edges of the lesion. Silver and immunocytochemical staining subsequently reveal neuritic regeneration associated with the bridges. Electron microscopy further reveals tubular shaped bridges consisting of regenerating neurites, myelinating Schwann cells, occasional macrophages and astroglia, blood vessels, fibroblasts and collagen. The time course of appearance and the arrangement of the fibroblasts and Schwann cells in relation to the neurites resembles conditions observed in peripheral nerve regeneration. Since the cellular substratum in peripheral nerves is an effective pathway of trophic support and chemotactic guidance for regenerating peripheral or central neurites, the cellular bridges could afford a means for central regeneration. To test this, we injected unconjugated type V HRP rostral to the lesion site of saline or LPS treated rats and examined spinal nuclei and dorsal root ganglia caudal to the lesion. Preliminary results showed that in the LPS treated rats there were more labeled cell bodies in spinal nuclei and dorsal root ganglia, indicating that both central and peripheral regenerating neurites are present in the lesion.

479.12

ALTERATIONS IN ROTATIONAL AND OPEN-FIELD BEHAVIOR FOLLOWING 6-OHDA LESIONING OF THE SUBSTANTIA NIGRA AND ADMINISTRATION OF ORG 2766. F.J. Antonawich and F.L. Strand. Department of Biology, New York University, N.Y., N.Y. 10003

ACTH peptide fragments that have little or no corticotropic activity have potent neurotrophic effects on peripheral nerves in situ and central neurons in culture. Unilateral lesioning of the substantia nigra, which depletes the striatum of dopamine, has been shown to alter unidirectional behavior in rats, which provides a feasible model for central regeneration. Male Sprague Dawley rats were lesioned unilaterally with 6-hydroxydopamine (8µg/4µl), infused into the substantia nigra over a ten minute period, resulting in unilateral Parkinsonism. They were subsequently treated with 10 µg/kg i.p. of ORG 2766 (ACTH 4-9 analog) or saline every 24 hours starting immediately after the infusion. Rotational behavior was analyzed using apomorphine (0.5 mg/kg) or amphetamine (2mg/kg) every 48 hours, with a challenge injection schedule organized to observe any compensatory mechanisms involved in recovery. Open-field behavior was also observed, looking for changes in grooming, rearing and exploratory behavior.

Preliminary rotational behavior analysis shows that ORG 2766 accelerates apomorphine-induced rotation to the contralateral side, suggesting the acceleration of a compensatory mechanism; however, amphetamine-induced rotation in ORG 2766 treated animals has, of yet, shown no difference from their saline counterparts. In open-field behavioral analysis, a trend is developing, over time, which shows a greater number of rearing episodes in ORG 2766 treated animals as compared to saline, with no apparent difference in the number of grooming and exploratory episodes.

479.13

PLASMINOGEN ACTIVATOR AND SCHWANN CELL PLASTICITY IN PERIPHERAL NERVE REGENERATION. N. Kalderon. The Rockefeller University, New York, NY 10021.

Plasminogen activator (PA) is a key enzyme controlling extracellular proteolytic activities. Mammalian cells produce 2 molecular forms of PA, urokinase type (u-PA) and tissue type and at least 2 types of PA inhibitors (PAIs), PAI-1 and PAI-2. The u-PA in concert with PAIs regulates cell migration and related tissue plasticity events. Undifferentiated Schwann cells express high PA activity levels (Kalderon, 1984, PNAS, 81:7216) primarily of the u-PA type (Kalderon, 1990, in Regulation of Extravascular Fibrinolysis in Nervous System Development & Disease, Plenum).

We are examining whether these plasticity properties of Schwann cells are instrumental in their support of nerve regeneration; the effects of protease inhibitors on rat sciatic nerve regeneration through a 10mm silicone chamber are being studied (Kalderon et al. 1987, SN Abstr 13:1208). The nerve stumps are sutured to a silicone tubing which is filled with a physiological salt solution. The inhibitors are injected into the chamber at 7 days postsurgery when a massive cell migration ensues, and their effect on nerve regeneration (Schwann cell migration and axonal growth) was examined a week later. Reported here, PAI-1 (1µg/ml) and aprotinin impaired cell migration at 52% and 48%, respectively, as compared with control samples; accordingly, axonal regrowth was delayed. These results and previous data (Kalderon et al., 1987) suggest that PA and plasmin activities are elaborated by the Schwann cells for migration and are essential for successful nerve regeneration.

479.14

TESTOSTERONE EFFECTS ON AXONAL REGROWTH FOLLOWING FACIAL NERVE INJURY. K.A. Kujawa and K.J. Jones. Dept. Cell Biology and Anatomy The Chicago Medical School, North Chicago, IL 60064.

We have previously demonstrated that testosterone propionate (TP) accelerates functional recovery from facial paralysis induced by facial nerve injury in castrated male hamsters. In this study, we tested the hypothesis that TP accelerates the regrowth of axons following facial nerve injury. Male hamsters were received as castrates from the supplier. Following anesthetization, the animals were each subjected to a right facial nerve crush, with the left side serving as internal control. One-half the animals immediately received subcutaneous implants of TP, with the others sham-implanted with blank capsules. Postoperative times ranged from 3-7 d. 18 h prior to sacrifice, stereotaxic injections of ^{3}H-amino acids into the right facial nuclear groups were done. Following sacrifice, the radioactivity in 1-mm segments of the right and left facial nerves was determined, and plotted as a function of postoperative time. The furthest distance point at which the radioactivity was > 2 S.D. above background levels defined the outgrowth distance of the fastest growing axons. The percentage of total radioactivity of nerve segments was also calculated and a profile of labeling of the entire population of regenerating axons obtained. The results indicate that TP accelerates the regrowth of both the fastest growing axons and the more slowly growing fibers which represent the bulk of the regenerating nerve. We are currently examining whether this acceleration of axonal regrowth involves an effect of TP on regeneration rate, delay of sprout formation or both.

479.15

TESTOSTERONE EFFECTS ON RIBOSOMAL RNA LEVELS IN REGENERATING HAMSTER FACIAL MOTONEURONS. N.B. Kinderman and K.J. Jones. Dept. Cell Biology and Anatomy,The Chicago Medical School, North Chicago, IL 60064.

We have previously demonstrated that testosterone propionate (TP) accelerates functional recovery from facial paralysis induced by facial nerve injury in castrated male hamsters. In this study, we tested the hypothesis that the effects of TP on nerve regeneration occur at the level of the neuron and through a mechanism involving priming of the genome. Analysis of the effects of TP on rRNA levels was accomplished using *in situ* hybridization and rDNA probes. Male hamsters were received as castrates from the supplier. Following anesthetization, the animals were each subjected to a right facial axotomy (AX), with the left side serving as internal control. One-half the animals immediately received subcutaneous implants of TP (TP + AX), with the others sham-implanted (AX only). Postoperative (PO) times ranged from 30' to 14 d. At the appropriate PO time, the animals were sacrificed by ether overdose and decapitation, and the brains rapidly blocked and frozen. Standard *in situ* hybridization and autoradiographic procedures were done on 8-µm, postfixed sections using a ^{3}H-28S rDNA probe. Computerized image analysis was employed for data collection. For both TP + AX, and AX only groups, the rRNA levels were expressed as a percentage of left side control. The results indicate that TP accelerates the initial rRNA increases observed with AX alone, as well as producing a dramatic effect on the magnitude of the response. Supported by an SCRF grant (KJJ).

479.16

REINNERVATION OF MEDIAL CORTEX BY CHOLINERGIC PROJECTIONS FROM BASAL FOREBRAIN: AN *IN VIVO* MODEL TO TEST HYPOTHESES ABOUT REGENERATION WITH GROWTH FACTORS. T.W. Farris and L.L. Butcher. Dept. of Psychology, UCLA, Los Angeles, CA 90024-1563.

To determine further the regenerative effects of putative growth-promoting factors on cholinergic projection neurons (previously assessed for GM1 ganglioside and estradiol), we administered thyroxine (T4, 2.5 mg/kg, ip) or saline daily for 14, 30 or 60 days to 8 week-old female rats with unilateral knife-cut axotomies of the medial cholinergic pathway arising from the cholinergic basal nuclear complex (CBNC) and projecting to cingulate and occipital medial cortices. Brain tissue was processed histochemically for acetylcholinesterase (AChE); immunohistochemically for choline acetyltransferase (ChAT), dopamine β-hydroxylase (DBH) and nerve growth factor receptor (NGFr); and for Nissl substance. Computerized densitometry performed via light microscopy showed, in both groups, the expected accumulation and depletion of all markers proximal and distal, respectively, to the cortical cut. However, the rate of recovery from distal depletion was significantly greater in T4-treated rats. Fluoro-gold placed distal to the cut revealed cell bodies in the vertical limb of the diagonal band and magnocellular preoptic nucleus. It was possible to directly assess axonal regeneration by observing fibers crossing the cut, as occurred in a few cases in 30 and 60 d T4 rats. These data (1) support previous findings from this laboratory that CBNC neurons are sensitive to thyroid hormones and (2) demonstrate that medial pathway axotomy is a useful model for determining whether factors known to possess growth-promoting activity *in vitro* induce axonal regeneration *in vivo* . [Support: NIH NS 10928]

THE AGING PROCESS: CELL BIOLOGY, MORPHOMETRY, OTHER

480.1

MAP-2 AND TUBULIN DEGRADATION BY CATHEPSIN D. J.M. Litersky, G.V.W. Johnson, and J.N. Whitaker. Depts. of Neurology, Psychiatry, Cell Biology Univ. of Alabama and the VA Medical Center, Birmingham, AL 35294.

The proteolysis required for the normal turnover of neuronal cytoskeletal proteins, such as MAP-2 and tubulin is poorly understood. Cathepsin D (EC 3.4.23.5), an aspartyl endopeptidase present in lysosomes and endosomes is abundant in many neurons. In this study, the in vitro degradation of MAP-2 and tubulin by cathepsin D was measured at various enzyme-to-substrate ratios and pH conditions using quantitative immunoblot techniques. MAP-2 showed a much greater sensitivity to cathepsin D than tubulin. At pH 3.5 and a 1:20 enzyme-to-substrate ratio, MAP-2 was totally degraded after 20 min, whereas tubulin under the same conditions was only 35% degraded after 20 min, and showed little additional substrate loss even after 60 min. The pH dependence of the cathepsin D-induced hydrolysis of MAP-2 and tubulin was also very different. At pH values between 3.0 and 5.0 the rate and extent of cathepsin D-mediated MAP-2 hydrolysis was not significantly different, however at pH 5.5 there was significant inhibition and at pH 6.0 only a small amount of the MAP-2 was degraded even after 60 min. In contrast, the hydrolysis of tubulin by cathepsin D was optimal at pH 4.5. At pH values of 3.0, 3.5, 5.0 and 5.5, tubulin was degraded by cathepsin D, but at significantly decreased rates, at pH 6.0 there was no significant hydrolysis of tubulin.

These results demonstrate that MAP-2 and tubulin are unequally susceptible to degradation by cathepsin D. These data also imply a potential for rapid degradation of MAP-2 by cathepsin D either in lysosomes, or in endosomes of the neuron. It appears unlikely that tubulin is a major substrate for cathepsin D. An understanding of these degradative pathways may contribute to our understanding of the processes involved in neurodegenerative diseases such as Alzheimer's disease, where abnormal processing of cytoskeletal proteins may be a contributing factor.

Supported by NIH grants #NS27538 and AG06569, the Alzheimer's Association/Mary Sue Glover Memorial Pilot Research Grant and the Research Program of the Veterans Administration.

480.2

AGE RELATED CHANGES IN *c-fos* EXPRESSION IN MICE. A.D'Costa, R.M. Booze, C.R. Breese*, R.L. Boyd, and W.E. Sonntag. Department of Physiology and Pharmacology, Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, NC 27103.

c-fos belongs to a class of genes that encode for nuclear proteins presumed to participate in the regulation of cellular growth and differentiation. It is thought to act as a "third messenger" molecule in signal transduction systems and its expression has been shown to be induced by a variety of exogenous and endogenous stimuli. Recent data indicate that the expression of the gene is repressed in senescent human fibroblasts (T. Seshadri et al., Science:247, 1990) which suggests a possible involvement in the mechanism of aging at the cellular level.

This experiment examines the differential expression of *c-fos* in various brain areas after a single electroconvulsive shock in four, eleven and twenty-five month old C57/BL6 mice. The animals received an acute electroconvulsive shock (90V for 0.2s), without prior anesthesia, through earclip electrodes. All animals exhibited generalized tonic-clonic seizures lasting 35-40s. An hour after the seizure, animals were anesthetized and perfused with 4% paraformaldehyde. The brains were Vibratome-sectioned (50 microns) and examined using *c-fos* antibody (1:1000;CRB), directed against a conserved region of both mouse and human *c-fos*, by standard ABC immunocytochemical methods. *c-fos* immunostaining was evident in the amygdala, granular layer of the dentate gyrus of the hippocampus and in the superficial layers of the cortex in the young animals. The oldest group showed a decrease in *c-fos* expression in these areas relative to the young animals, whereas the middle aged animals exhibited no difference. This reduced induction of *c-fos* in aging animals may serve as an indicator of brain aging. (This work was supported by NIH grant AG 07752 to W.E. Sonntag).

480.3

DISTINCT FORMS OF THE ALZHEIMER'S AMYLOID PRECURSOR ARE PRESENT AT HIGHER LEVELS IN FETAL THAN IN ADULT HUMAN CORTEX. K. Sambamurti*, M. Mohamadi*, J.P. Anderson*, P. Knott and N.K. Robakis, Mount Sinai Medical Center, One Gustave Levy Place, New York, N.Y. 10029.

The ß/A4-amyloid protein, which is the major component of the neuritic plaque that accumulates in the brains of Alzheimer patients, derives from a larger precursor protein (ßAPP). We have used antisera raised against a series of peptides derived from ßAPP to study the various forms of the precursor in adult and fetal cortex. Two independent antisera specific to the extracellular domain (R3 and R5) detected three bands in brain extracts migrating at 98, 105 and 110 KD which were present at significantly higher levels in fetal than in adult brain. This difference was not due to masking of antigenic sites in the adult brain due to either phosphorylation or glycosylation. In contrast, and in agreement with published data, an antibody against the cytoplasmic domain (R1) detected bands in the range of 110 to 120 KD were not significantly different in the fetal and adult brain. Concomitant with the loss of the three ßAPP bands, two new protein bands of approximately 50 KD and 40 KD were detected in adult but not fetal brains. All these bands were specific as demonstrated by peptide competition. These results suggest that certain forms of ßAPP are developmentally regulated.

480.4

REDUCTION OF RAT BRAIN SOMATOSTATIN mRNA EXPRESSION DURING AGING. T. Florio, C. Ventra, O. Meucci*, A. Scorziello*, and G. Schettini. Dept. of Pharmacology, II Medical School, Univ. of Naples, via S. Pansini 5, 80131 Naples, Italy.

In the past years, a growing bulk of studies demonstrated that the blockade of central somatostatin neurotransmission caused an impairment of learning and memory processes in the experimental animals. Moreover, clinical studies reported that, in patients affected by the dementia of the Alzheimer's type, the somatostatinergic neurotransmission is quite affected. Thus, a primary role for the brain somatostatin in the modulation of cognitive functions has been suggested. In this report we studied whether an alteration in the brain somatostatinergic neurotransmission "naturally" occur during the aging process in the rat. For this purpose we analyzed the somatostatin mRNA expression in different rat brain areas (frontal cortex, parietal cortex, hippocampus, striatum) derived from 2, 6, 12, and 25 months old rats, by means of both northern and dot blot analysis, using pre-prosomatostatin cDNA (kind gift of Dr. R. H. Goodman) as probe. A clear reduction of somatostatin mRNA expression occurred in both frontal and parietal cortex in the aged rats.

480.5

EXPRESSION OF HEAT SHOCK mRNAs IN AGED RAT BRAIN. J.D. Raese, S. Pardue*, K. Groshan*, E.K. Miller*, B. Border, R. Gonzales* and M. Morrison-Bogorad. Departments of Psychiatry, Neurology and Biochemistry, University of Texas Southwestern Medical Center, Dallas, Texas, 75235, and The Schizoprenia Research Center, V.A. Medical Center, Dallas, Texas, 75216.

mRNAs encoding strictly heat inducible (hsp70) and constitutively-expressed (hsc70) members of the heat shock 70 gene family show different cell distributions in adult rat brain. Nothing is known about possible alterations in the heat shock response in different cell types of aged brain. We compared in situ hybridization of oligos specific for the hsp70 and hsc70 mRNAs in cerebellum and hippocampus of 4 adult and 3 aged rats, each subjected to a heat stress of identical duration and intensity. Rats were sacrificed 3 hours after initiating the heat stress. Grain counts in each cell type in different animals were normalized by hybridization of adjacent sections to an 18S rRNA oligo. In 3/4 adults, hsp70 mRNA induction was several fold greater in granule cells of the dentate gyrus than in granule cells of the 3 aged rats. In the adult rats, hsp70 mRNA levels were also higher in CA1 and CA4 pyramidal neurons. There was a very strong glial response in both age groups. Hippocampal hsc70 mRNA distribution was very different. This mRNA was abundant in neurons and very low in glia. Relative levels of hsc70 mRNA varied in different animals but there was no consistent difference between adult and aged rats or between the relative levels of either mRNA in cerebellar cells of either age group. However, hybridization with the 18S oligo showed that levels of 18S rRNA were lower in Purkinje cells of aged rats. These results show that there are age-related changes in the stress response of hippocampal neurons. Such changes may predispose these cells to stress-related injury or death. Funded by NIH AG08013 and HD14886, and by the Leland Fikes Foundation.

480.6

MODULATION OF TYROSINE HYDROXYLASE GENE EXPRESSION IN THE RAT ADRENAL GLAND BY RESERPINE: EVIDENCE FOR UNCOUPLING OF TRANSCRIPTIONAL AND POSTTRANSCRIPTIONAL MECHANISMS DURING AGING. R. Strong, C. Hale, M. A. Moore and M. Wessels-Reiker. Departments of Pharmacology and Medicine, St. Louis Univ. Sch. Med. and GRECC, VA Med. Cntr. St. Louis, MO 63125

Plasma catecholamine levels are increased during aging. Because tyrosine hydroxylase (TH) is the rate limiting enzymatic step in catecholamine synthesis, we are studying alterations in the regulation of TH gene expression during aging. Tyrosine hydroxylase, TH messenger RNA (TH mRNA) and dopamine (DA) were measured simultaneously in adrenal glands of individual Fischer 344 rats aged 2, 6, 13 and 23 months. Between 2 and 23 months TH activity rose 2 fold as compared to the youngest group. TH mRNA content of the adrenal gland rose 3 fold between 2 and 23 months. A 3 fold increase in adrenal DA content, the first catecholamine product of TH, provides evidence that the increases in TH gene expression are functionally significant.

To determine if mechanisms that regulate gene expression are altered by aging, the effects of reserpine on induction of TH mRNA and TH activity were compared in another group of rats aged 2, 12 and 27 months. Consistent with the results of the first experiment, there were age-related increases in both TH activity and TH mRNA in the age-matched control groups. TH activity rose 2 fold and TH mRNA rose more than 6 fold between 2 and 27 months. The discrepancy in the relative magnitudes of increases in TH mRNA and TH protein suggest an uncoupling of regulation of TH mRNA and TH protein levels. Moreover, there were significant age-related differences with respect to modulation of TH gene expression by reserpine treatment. TH activity was induced by reserpine in the youngest group, but not in the two older age-groups. In contrast, reserpine caused significant induction of TH mRNA in all age-groups. These results provide evidence that aging is accompanied by alterations in TH gene expression at transcriptional and post-transcriptional levels.

480.7

EFFECTS OF AGING AND DENERVATION ON ADRENERGIC RECEPTORS OF THE CEREBRAL CORTEX AND CEREBRAL MICROVESSELS IN RATS. S.I. Harik, S. Sromek* and R.N. Kalaria. Dept. of Neurology, Case Western Reserve Univ. Sch. of Med., Cleveland, OH 44106.

Locus ceruleus (LC) lesions cause cerebral norepinephrine loss and increased density of ß adrenoceptors in the cortex and its microvessels (mv). This "denervation supersensitivity" implies that these receptors are responsive to LC input. We studied the effect of aging and denervation on these adrenoceptors in 3 age groups of Fisher-344 rats: young (3-4 months), middle-aged (12-13 months), and old (25-26 months). Unilateral LC lesion was induced by microinjection of 4 µg of 6-hydroxydopamine. Two weeks later, the rats were killed and samples from each cerebral cortex were assayed for norepinephrine and for adrenoceptor assays. The rest of cerebral cortical mantles from ipsilateral and contralateral cortices were pooled from 5-6 rats and their mv harvested and assayed for α_1 and ß adrenoceptors using [^{125}I]-HEAT and [^{125}I]-pindolol. We found that ß receptor density increased by about 50% in both cortex and mv ipsilateral to lesion in all age groups, but there were no significant changes in α_1 receptors. Also, aging had no effect on the density of both adrenoceptors in the contralateral cortex and mv.

480.8

VESICULAR FAST AXONAL TRANSPORT RATES FOR RAT SCIATIC NERVE AXONS AS A FUNCTION OF ANIMAL AGE. N.A. Kreiter* and T.A. Viancour. Department of Biological Sciences, University of Maryland, Baltimore County Campus, Catonsville, MD 21228.

We report the results of a pilot study of vesicle transport in aging axons. Longitudinally moving vesicles in dissected, myelinated axons from rat (Fisher 344 strain) sciatic nerves were observed by high resolution, video-enhanced-contrast microscopy at an experimental temperature of 37 ± 0.2°C. Transport rates of individual vesicles were determined by measuring the transit time through a 2-3µm long speed trap which was electronically superimposed on the video image of the moving vesicles. Transport rates of more than 8,000 vesicles were measured for both a young (ca. 8 wks) and an old (ca. 2 yr) age group.

Under these conditions, average transport rates of both anterograde moving vesicles and retrograde moving vesicles were significantly slower in the older animals ($p << 0.001$, Student's T-test). Among the yet to be tested explanations of these data is the possibility that vesicle movements are impeded by age-dependent properties of the axonal cytoskeleton.

Transport Direction	Young Age Group avg ± SEM (n)	Old Age Group avg ± SEM (n)
Anterograde (µm/s)	3.02 ± 0.02 (3765)	2.41 ± 0.03 (2012)
Retrograde (µm/s)	2.89 ± 0.01 (5439)	2.50 ± 0.01 (6166)

(We thank Don Ingram and David Clissold for their contributions. This pilot study was funded by the UMGSB SRIS program.)

480.9

SEGMENTAL DEMYELINATION IN HINDLIMB NERVES OF AGED CATS. A.M. Adinolfi, J.Yamuy, F.R. Morales and M.H. Chase. Dept. of Anatomy & Cell Biology, Dept. of Physiology and Brain Research Institute, UCLA School of Medicine, Los Angeles, CA. 90024.

This study of the ultrastructure of sciatic nerve branches in normal old cats provides evidence that segmental demyelination is one cause for the decrease in old age in the mean axonal conduction velocity of hindlimb nerves. Eight cats, one to 17 years old, were prepared for peripheral stimulation of hindlimb nerves and intracellular recording from lumbar motoneurons. Samples of unperturbed nerves from the opposite hindlimb were processed for electron microscopy. The mean conduction velocity, calculated by dividing conduction distance by the latency of the antidromic spike, slowed from 102.8 m/s±10.8 (SD) at one year to 70.6 m/s±7.9 (SD) in the oldest cats. In these animals, 8-12 % of nerve fibers showed focal abnormalities of the myelin sheath. These changes included 1) lipoid and granulo-vacuolar debris in distended inner adaxonal and outer cytoplasmic parts of the Schwann cell, 2) retraction of paranodal myelin loops to widen nodes of Ranvier and 3) disruption of one or more contiguous segments of the myelin sheath by intralamellar ballooning along major dense and intraperiod lines. (Supported by USPHS AGO 4307.)

480.10

BLOOD-NERVE BARRIER PERMEABILITY AND NERVE VASCULAR SPACE IN THE PERIPHERAL NERVE OF RATS OF DIFFERENT AGES. J. Koistinaho, K.C. Wadhwani, A. Balbo and S.I. Rapoport. Lab. of Neurosciences, NIA, NIH, Bethesda MD 20879. The permeability-surface area products of (14C)-sucrose at the blood-nerve barrier, PA(BNB), of the sciatic nerve; and at the blood-brain barrier, PA(BBB), were determined in Fischer-344 rats at 3, 11 and 30 mo of age, using an in vivo i.v. bolus injection technique and a two-time point graphical method. Nerve vascular space in the tibial nerve of these rats was also determined using quantitative morphometry. There was no significant difference of mean PA(BNB) between any age group [PA(BNB) at 3 mo=1.1±0.1(SE), at 11 mo=1.8±0.3; and at 30 mo=1.4±0.2 x10(-5) ml/s.g wet wt; n=5-8 rats], nor any difference in PA(BBB). The mean ratio of surface area of endoneurial blood vessels/nerve cross section (%) of the tibial nerve also did not differ between any group [3 mo: 16±2 vessels; mean surface area ratios =2.11±0.10 %, n=6; 11 mo= 22±3 vessels and 2.48±0.21 %, n=5; and at 30 mo= 26±1 vessels and 2.40±0.23 %, n=4). Our results indicate that BNB and BBB integrities are not altered in senescent Fischer-344 rats.

480.11

UNBIASED STEREOLOGICAL ESTIMATION OF THE TOTAL NUMBER OF NEURONS IN THE AGING HUMAN HIPPOCAMPUS

M. J. West and H.J.G.Gundersen*

Stereological Research Lab., Univ. of Aarhus, Denmark

The total numbers of neurons in the dentate granule cell layer, the hilus, regio-inferior, regio superior and subiculum were estimated with unbiased stereological techniques and systematic sampling in 16 patients ranging from 15 to 85 years of age. Total neuron number was calculated as the product of the numerical density, N_V, and the reference volume, V_{ref}, of the layer containing perikaria. N_V was estimated with optical disectors, V_{ref} by point counting. Sampling in both cases was carried out systematically, i.e., with a random start and a predetermined periodocity. The sampling scheme was designed so that the major contribution to the variance of the group was the true biological difference amoung individuals. Preliminary data indicates that is there a significant correlation between age and neuron number in regio superior. This is an inverse relationship indicating a loss of 50% of the neurons in regio superior, but not in other hippocampal subdivisions, during adult life.

J. Comp. Neurol. 296: 1-22, 1990

480.12

AGE-RELATED REGRESSIVE CHANGES IN MOTONEURON NUMBER AND MORPHOLOGY IN AN ANDROGEN-SENSITIVE RAT SPINAL NUCLEUS. E.M. Kurz and D.R. Sengelaub. Program in Neural Science, Dept. of Psychology, Indiana University, Bloomington, IN 47405.

Motoneurons in the sexually dimorphic spinal nucleus of the bulbocavernosus (SNB) in rats are sensitive to androgens during development and at adulthood. Developmentally, androgens regulate motoneuron number, soma area, and dendritic morphology. In adults, reductions in androgens after castration produce significant declines in SNB soma area and dendritic length, and these can be restored by androgen treatment. Androgen titers decline during normal aging in male rats, and testosterone titers in aged males are about 1/3 of young adult levels. We examined whether this age-related, naturally occurring decline in androgens altered SNB motoneuron number, soma area, or dendritic length.

SNB motoneuron number and morphology were assessed in aged (22 months old; n=5) and young adult (70 days old; n=4) males after retrograde labeling following unilateral muscle injection with cholera-toxin HRP and counterstaining with thionin. Aged males had significantly fewer SNB motoneurons than young adults (144 *vs.* 172); counts of HRP-labeled motoneurons were also lower in aged males (45 *vs.* 51). HRP-labeled SNB motoneurons in aged males were significantly smaller than those of young adults in both soma area (760 *vs.* 843 sq μm) and dendritic length (1891 *vs.* 4114 μm). Weights of the androgen-sensitive SNB target muscles (bulbocavernosus/levator ani) were also significantly lower in aged males (0.92 *vs.* 1.23 g). These data suggest that age-related regressive changes in SNB motoneuron number, soma area, dendritic length, and target muscle weight may result from declining androgen levels. (Supported by NIH NS24877)

480.13

SEX- AND AGE-RELATED DIFFERENCES IN MIDSAGITTAL AREAS OF THE CEREBRAL CORTEX AND CORPUS CALLOSUM: AN MRI INVESTIGATION. N. Raz, W. Spencer, and I. Torres. Dept. Psychology, Memphis State University, Memphis, TN 38152. We examined sex- and age-related differences in the area of the cerebral cortex (Cx), corpus callosum (CC), and subdivisions of the CC. Midsagittal magnetic resonance (MR) images were obtained from a sample of 51 normal volunteers and pseudoneurological controls (age 17 - 87). Males had larger Cx, CC, and splenial areas than females [t(49)=6.63, p<.001, t(49)=2.07, p<.05, and t(49)=2.74, p<.01, respectively]. However, the correlations between the areas of CC and splenium, and Cx were also significant (r=.28, p<.05; r=.33, p<.02, respectively). The Cx area is an estimate of the brain size, and as such is influenced by gender differences in body size. After controlling for this index of brain size, we found no significant effects of gender on CC or splenium area. The Cx area was weakly associated with age (r=-.32, p<.02), but no age-related differences were found in CC area or its subdivisions. Supported by BRSG S07-RR0-5366-26 and MSU Center for Applied Psychological Research.

480.14

MAGNETIC RESONANCE IMAGES OF THE CEREBRAL INFARCTION OF AN AGED RHESUS MACAQUE. H. Uno, W.D. Houser*, and J.E. Holden*. Wisconsin Regional Primate Research Center and Medical Physics, University of Wisconsin, Madison, Wisconsin 53715-1299.

Magnetic resonance imaging of a 30 year old, male rhesus macaque suffering from ataxia of the bilateral fore and hind limbs for approximately one year was performed at 6 month intervals. After the 2nd test, the animal was euthanized due to severe locomotive failure. The formalin-fixed whole brain was also examined by MRI, then sliced into 5 mm thicknesses along the horizontal plane. Scanning was performed in a 1.5 tesla (GE) unit. The images of horizontal, coronal, and sagittal planes were obtained using a Tr (repetition rate) = 2000 msec and either TE (echo time) = 20 msec for spin density or TE = 90 msec for 2nd echo images. In the 2nd echo (T_2 dependent) images, irregular opaque shadows were found in the left inferior parietal lobe along the lateral sulcus, right parietal lobes and bilateral cerebellar cortex. However, the images of spin density in pre- and post-mortum brain confirmed that the opaque shadows showing in T_2 images were caused by CSF accumulation in widened meningeal spaces associated with a defect of the cortex (old infarction). Pathology of the brain showed multiple infarctions (old and liquified lesions) in the above regions. Different relaxation times [CSF = water (400-450 msec); brain cortex (250-300 msec)] produced the contrasting grey tone in spin density and 2nd echo (T_2); e.g. CSF, black in the former and white in the latter and brain cortex, vice versa. A combination of different Tr and TE times is necessary to determine the real nature of pathological lesions.

480.15

Aging of the Human Brain: Assessment with 1.5 Tesla MRI

P. Murali Doraiswamy, Gary Figiel, Mustafa Husain, Orest Boyko, William McDonald, Sunjay Shah, Everett Ellinwood, and Ranga Krishnan. Duke University Medical Center and Washington University School of Medicine, St. Louis, MO.

Brain MR images of 39 normal volunteers, aged 26 to 79 yrs were used to assess neuroanatomical and biophysical changes in the aging brain. Axial MR images revealed a significant increase in the frequency of subcortical hyperintensities in older subjects. Older subjects also demonstrated significantly smaller caudate and putamen volumes compared to younger subjects. Sagittal images revealed significant age-related dimensional changes in the corpus callosum, pituitary gland, midbrain, septum pellucidum and cortex. Coronal multi TR/TE images showed a significant increase in T1 relaxation times in the hippocampus, corpus callosum, frontal and temporal white matter in the older subjects when compared with younger subjects.

These data represent the first in vivo study to simultaneously document anatomical as well as biophysical tissue changes in specific regions in the human brain in normal aging. Neurocognitive studies are underway in these subjects to evaluate the potential functional correlates of these findings and to establish structure-function relationships in the aging human brain.

480.16

BIOMARKERS OF HIPPOCAMPAL AND RETINAL AGING ARE NOT ALTERED BY DIETARY RESTRICTION. W.K. O'Steen, L.B. Cadwallader*, S. Vinsant*, and P.W. Landfield. Depts. Neurobiol. & Anat. and Physiol. & Pharmacol., Bowman Gray Sch. Med., Wake Forest Univ., Winston-Salem, NC 27103.

Dietary restriction is one of the few established methods for prolonging life span in mammals, and development of several biomarkers of aging has been found to be slowed by prolonged restriction. However, much less is known about the effects of restriction on markers of brain aging. In the present studies, therefore, we have investigated the effects of long-term dietary restriction (60% of ad lib diet, beginning at 16 weeks of age) on quantitative morphometric measures of aging in the hippocampus and the retina of Fischer 344 male rats. The animals were maintained by the NIA Biomarkers program at the Nat. Center Tox. Res. Each experimental group ranged in size from 8 to 16 rats.

A gradual thinning of the retinal outer nuclear layer of photoreceptor nuclei occurs with aging in the control ad lib groups. However, the restricted diet did not influence retinal aging in 18, 21, or 27 mo-old rats, as judged by photoreceptor cell death, outer nuclear layer thickness, and pattern of cell loss.

Hippocampal pyramidal cell density and astrocytic inclusions correlate closely with aging in F344 rats. In two studies, no differences in hippocampal neuronal density were found between *ad lib* (AL) and dietary restricted (DR) animals in the older groups (26-27 mo-old). However, in the 18 mo-old and 21 mo-old animals, a small (about 10%), but significant difference was found, with DR animals exhibiting higher neuronal density. In 26 mo-old rats, no significant effects of dietary condition were found on quantitative E.M. measurements of glial inclusions, neuronal lipofuscin, rough endoplasmic reticulum or Golgi apparatus.

These results are in contrast to studies of restriction on non-neuronal markers of aging, including basic longevity, and suggest that a different aging mechanism may modulate at least some aspects of brain aging. (Supported by AG07767)

480.17

EFFECT OF LIFELONG VOLUNTARY ETHANOL CONSUMPTION ON AGING IN RATS. K. Kiianmaa[1], M. Sarviharju[1]*, P. Jaatinen[2]*, P. Salmi[2]*, A. Hervonen[2]. [1]Res. Labs., Alko Ltd., 00101 Helsinki, and [2]Lab. of Gerontology, Univ. Tampere Med. School, 33101 Tampere, Finland.

The effect of 20 months of voluntary ethanol consumption on aging was studied in several generations of high drinking AA (Alko alcohol) rats. After the initial test of individual voluntary ethanol intake at the age of three months the rats were housed in group cages. One half was given free access to food, tap water, and 10 % (v/v) ethanol solution, while the other half had only food and water available. The survival curves of both ethanol exposed and ethanol non-exposed groups were monitored. Several generations were pooled together or compared within the generation. No changes were found in the population longevity up to the age of 24 months when the rats were used for experiments. The ethanol exposed group consumed slightly more ethanol than the non-exposed group when measured at the age of 24 months. They did not show any difference in ethanol sensitivity when tested for ethanol-induced (2 g/kg ip) motor impairment on the tilting plane, for the duration of ethanol-induced (3.5 g/kg ip) loss of righting reflex, or for ethanol-induced (3.5 g/kg ip) hypothermia. The groups did not differ in the rate of ethanol elimination either. Determination of the concentrations of brain monoamine neurotransmitters and their major metabolites did not reveal any differences between the ethanol exposed and non-exposed old rats, while the levels tended in both groups generally to be higher than in 3-4 month old rats. Dendritic spine counts after Golgi-stain were not different either. The ethanol-feeding regimen used thus did not produce any measurable negative interactions of ethanol on aging.

480.18

AGE-DEPENDENT CHANGES IN VISUAL INTENSITY DIFFERENCE THRESHOLD IN PIGEONS. M. Kurkjian* and W. Hodos. University of Maryland, College Park, MD 20742.

Recent studies have reported age-related deficits in visual acuity and retinal morphology in pigeons. The study reported here was designed to determine the effects of age on intensity difference thresholds (IDT) in pigeons. 76 pigeons, aged 1-17 years, were trained to discriminate two stimuli that differed in luminance by 0.6 log unit. When this discrimination was established, the subjects were presented with two series of progressively smaller luminance differences. IDT was calculated from psychometric functions by determining the luminance difference that corresponded to 75% correct. The coefficient of correlation between age and IDT was +0.26, which indicates a small, systematic relationship between IDT and age; i.e., age accounts for approximately 5% of the variance in IDT. This is in contrast to visual acuity in pigeons in which age accounts for 82% of the variance. The results reported here, which are in agreement with human literature, indicate that age-related deficits in visual acuity and other visual tasks in pigeons are not due to a general deterioration of the visual system or to some non-specific global performance deficit.

480.19

AGING AND EYE MOVEMENT ON COPYING FIGURE. S. Murakami*, J. Miyazawa**, M. Fujii**, R. Fukatsu**, Y. Aizawa**, N. Nakano**, N. Takahata**, T. Fukuda***, M. Yamada***. Yamanoue Hospital*, Dept. of Neuropsychiatry, Sapporo Medical College, Sapporo, 060**. NHK Science and Technical Lab., Tokyo, 110***.

Eye movement, to move the eyes so that the point of interest will be seen with the visual center of the retina, tells us valuable information about a human higher brain function including visual cognition. In this experiment, we investigate the development time course of eye movement on copying a cube by using a new technique, Vision Analyser (TKK 939), experimented in 30 subjects (6-81 year-old).

In 6-7 years, eye velocity on copying was diffuse to a wide range and no peak, as developing the most used eye velocity was appeared and going slower. A mean eye velocity was 76.6 deg/sec in 6 years, 32.6 deg/sec in 25 years and 23.2 in 81 years old. On the other, total eye fixation time was going longer as developing, but it made a peak at 20-30 years old. And gazing points localized more on the model figure in young below 10 years old and on the copyed figure in older than 10 years.

The development time course of eye movement on copying a cube is obtained for the first time objectively, so these results indicate that a development of ability of visual cognition and hand writing due to visual information, is to increase until 20-30 years and hold on.

480.20

AGE-RELATED DECLINE OF NEURONAL RESPONSES TO SENSORY STIMULATION IN THE RAT SI VIBRISSA CORTEX. J.M. Greuel, W. Scheip*, K.M. Bode-Greuel, M. de Jonge, T. Schuurman*, T. Glaser, J. Traber*. Institute for Neurobiology, Troponwerke GmbH & Co.KG, Berliner Str.156, 5000 Köln 80, F.R.G.

Performance of aged rats in a number of behavioral tests is disturbed when compared to young rats. It has been hypothesized that altered brain functions contribute to the reduced performance. We investigated whether the aging process affects the processing of sensory information. Young (3-4 months) and aged (>32 months) rats were anaesthetized and the rat SI vibrissa cortex was exposed for single unit recording. Single whiskers were stimulated by using hand-held probes. The neuronal responses to the sensory stimulation were assigned to either of four classes according to their response vigour. It was found that in the aged rats less cells responded to the applied stimuli. Of those responding to the stimuli the neurons of the aged animals responded less vigourously, thus leading to a shift in the response quality distribution towards lower response qualities. Taken together, the results suggest i) that changes in cerebral function that may contribute to altered behavioral performance can be detected at the level of sensory processing and ii) that the vibrissa cortex of the rat can be a suitable model to study age-related changes in cortical function.

481.1

INCREASED PLASTICITY OF AGING MOUSE PECTINEUS NEUROMUSCULAR JUNCTIONS OBSERVED IN VIVO N. Robbins, J. Hill* and R. Hill*, Dept. Neurosciences, Case Western Res. Schl. of Medicine, Cleveland, OH 44106.

In order to delineate dynamic cellular processes underlying age changes at the neuromuscular junction (NMJ), living identified NMJ's were observed at intervals of 1 week or 1 month in adult (6 mo.) and old (27-29 mo.) CBF-1 mice. Fluorescent stains for pre- and post-synaptic elements were, resp., Texas Red-tetanus toxin C fragment (recombinant, Boehringer Mannheim, or proteolytic-derived) and FITC-alpha-bungarotoxin. At first observation, old NMJ's revealed more separate synaptic regions, more nerve terminal (NT) constrictions, and a greater area of NT outgrowth per NMJ than did adult NMJ's. Over 1 week (but not 1 mo.), more focal constrictions appeared and more pre-existing NT constrictions expanded in old NMJ. Over 1 month, NT outgrowth/retraction was greater in old NMJ, with 50% of old NMJ's (vs., 14% adult) showing 3 or more types of change, some markedly so. In sum, increased focal NT withdrawal may eventually produce the constrictions and multiple regions characteristic of old NMJ's, whereas enhanced in- and outgrowth of NT may account for increased abandoned postsynaptic folds. These dynamic events could result from decreased adhesion of NT to synaptic matrix and possibly increased perijunctional adhesion. Supported by NIH AG 06641.

481.2

LONG-TERM REGULATION OF TRANSMITTER RELEASE FROM CRAYFISH MOTOR TERMINALS BY DEPOLARIZATION OF THE MOTONEURON CELL BODY. S.J. Hong and C.A. Lnenicka. Dept. of Biol. Sci., State Univ. of New York, Albany, NY 12222.

Previous in vivo studies demonstrated that electrical stimulation of a crayfish phasic motor axon, proximal to a TTX conduction block produced long-term changes in transmitter release: less initial transmitter release, and greater fatigue-resistance (J. Neurophysiol. 61:91-96, 1989). In order to further examine this effect, the cell body of a phasic motoneuron was depolarized in vitro by current injection, and the physiology of its neuromuscular synapses examined. The cell body of the abdominal fast flexor motoneuron F3 was depolarized at a level subthreshold for impulse generation (30-40 mV; 30 mS duration) at 5 Hz for 2 hrs. At 5 hrs after this period of conditioning, the initial EPSP amplitude of the conditioned side (16.6 $\pm$ 2.0 mV) was significantly smaller than the control side (20.0 $\pm$ 1.8 mV, $p < .001$; $n = 17$). This reduction in initial EPSP amplitude (17%) is similar to the 25% reduction seen 24 hrs after in vivo central stimulation (J. Neurophysiol. 61:91-96, 1989). The present result shows that depolarization of the cell body can influence transmitter release from distant neuromuscular synapses.

481.3

SEASONAL CHANGES IN THE PHYSIOLOGY AND MORPHOLOGY OF IDENTIFIED CRAYFISH MOTOR TERMINALS. G.A. Lnenicka and Y. Zhao, Dept. of Biol. Sci., State University of New York, Albany, N.Y. 12222.

Seasonal changes in synaptic terminal physiology and morphology were studied in the crayfish fast closer excitor (FCE), a phasic motoneuron innervating the claw closer muscle. FCE EPSPs recorded from closer muscle fibers in summer animals showed a significantly lower initial EPSP amplitude (8.7 $\pm$ 1.5 mV) compared to winter animals (14.7 $\pm$ 1.7 mV, $p < .02$), and a significant increase in fatigue-resistance. During 30 min. of 5 Hz stimulation, FCE EPSP amplitude in summer animals decreased only 1%, while EPSP amplitude in winter animals decreased 82% ($p < .02$). In some animals, HRP was intracellularly injected into the FCE axon to examine terminal morphology, and it was found that the motor terminals in summer animals possess significantly more synaptic varicosities (.92 $\pm$ .10 varicosities/10 μm terminal) than those in winter animals (.40 $\pm$ .05 varicosities/10 μm terminal, $p < .001$).

The physiology and morphology of the FCE terminals in summer animals is similar to that seen after in vivo stimulation of the FCE in winter animals. It is proposed that high activity levels in summer animals are responsible for the seasonal change in the synaptic terminals.

481.4

EFFECT OF VARYING CALCIUM CONCENTRATIONS ON JUNCTION POTENTIALS RECORDED FROM NORMAL AND REPRESSED SYNAPSES IN THE CRAYFISH. A. Gupta* and S.J. Velez Dept. of Biological Sciences, Dartmouth College, Hanover, N. H. 03755

The synaptic connections of the neurons innervating the superficial flexor muscles of the crayfish Procambarus clarkii become repressed (no junction potentials are detected under normal physiological conditions) when the nerve is cut at the center of the muscle field and the lateral fibers are removed (Prosser & Velez, Soc. Neurosci. Abst. 12: 1575, 1986). We suggested that calcium ions could be involved in this repression. In the present work we study the effects that changes in calcium concentrations have on the junction potentials (jp's) recorded from normal and repressed animals. The same type of surgery was performed to obtain a pool of repressed animals, which were analyzed between three to five weeks after the operation (the peak time for repression). Analysis consisted of recording from medial muscle fibers with a microelectrode the jp's generated by the spontaneous firing of neuron 3. The microelectrode was left in the fiber while changing Ringers solutions which varied in calcium concentration from 30 to 200%. Normal animals showed a 60-90% increase in jp's sizes with increasing calcium concentrations while repressed animals showed only a 2-20% increase. Since repressed synapses do not respond to changes in calcium concentrations as normal synapses do, this suggests that calcium is indeed involved in the mechanisms underlying repression in this system.

481.5

DIFFERENTIAL ALTERATIONS IN VISCERAL SENSORY NEUROTRANSMITTERS OF THE NODOSE (NG) AND PETROSAL GANGLIA (PG) IN RESPONSE TO AXOTOMY. C.J. Helke and A. Rabchevsky*. Dept. of Pharmacol., Uniformed Services Univ., Bethesda, MD 20814.

Acute peripheral axotomy of the visceral sensory neurons of the vagus and glossopharyngeal nerves removes peripheral depolarizing and trophic influences to their sensory ganglia. Whereas transmitter expression is altered by axotomy of somatic sensory neurons, axotomy-induced transmitter changes in visceral sensory neurons are largely unexplored. To study this, rats were sacrificed 1, 3, 7 or 14 days after transection of either the cervical vagus or the glossopharyngeal nerves. The numbers of calcitonin-gene related peptide (CGRP) immunoreactive (ir), tyrosine hydroxylase (TH)-ir, and vasoactive intestinal peptide (VIP)-ir neurons in the respective ganglia (NG & PG) were analyzed in axotomized vs. control ganglia. In the NG, axotomy of the cervical vagus resulted in a rapid (by 1 d) reduction in the number of TH-ir cells. CGRP-ir cells were decreased at 7 and 14 days, whereas VIP-ir neurons were dramatically increased in number by 3 days. In the PG, axotomy of the glossopharyngeal nerve similarly reduced the TH-ir cells but had less effect on CGRP-ir cells and did not increase the number VIP-ir neurons. Thus, peripheral axotomy of visceral sensory neurons differentially changed the content of putative transmitters in their cell bodies. These data have implications for immunocytochemical-retrograde tracing studies with tracer application to cut axons and for studies of cultured visceral ganglia. (NS20991)

481.6

ULTRASTRUCTURE OF "SPROUTED" SAPHENOUS AFFERENTS IN THE RAT LUMBAR DORSAL HORN AFTER SCIATIC DEAFFERENTATION INDUCED BY INJECTION OF THE SCIATIC NERVE WITH PRONASE. A. El-Bohy, S. E. Kapadia, and C. C. LaMotte. Section of Neurological Surgery, Yale Univ. Sch. Med., New Haven, CT. 06510.

We have previously demonstrated expansion of the terminal field of the saphenous nerve 4 months following deafferentation by injection of the sciatic nerve with proteolytic enzymes (Pronase)(JCN, '89). In these experiments, the sciatic nerve was injected with 20mg Pronase; the opposite side served as a control. Four months following the injection, the saphenous nerves on both sides were injected with 0.5 mg WGA-HRP and .05 mg bCT-HRP, and the animal sacrificed after 48 hours. Sections demonstrating maximal expansion were prepared for EM, using the AHM (ammonium heptamolybdate) method for the peroxidase reaction. Low magnification EM montages were prepared, covering the labelled territory on the pronase and the control sides. All labelled terminals were located on the montage and rephotographed at 10K. In each animal, 70-100 terminals on each side were classified by location and by type (simple or glomerular), and the number of synaptic contacts made by each terminal counted. There was an increase in terminal number in all laminae on the pronase side, particularly laminae IIi and III. Quantitative analysis of lamina II revealed that there was an increase in the ratio of glomeruli/simple terminals on the pronase versus control side while the number of synapses per glomerulus was similar on both sides. (NIH grant NS 10174).

481.7

INCREASED LEVELS OF TYROSINE HYDROXYLASE (TH) AND NEUROPEPTIDE Y (NPY) IN CILIARY GANGLIA OF SYMPATHECTOMIZED RATS. Sophia Tyrrell, Ruth E. Siegel and Story Landis. Dept. Neurosci. and Pharm., Case Western Reserve Univ. Sch. of Med., Cleveland, OH. 44106.

The mechanisms that determine and regulate the expression of transmitters in different neuronal classes are incompletely understood. We have examined several neurotransmitter properties in the adult ciliary ganglion of control and sympathectomized rats. Although the ciliary ganglion is parasympathetic and functionally cholinergic, it has been shown to express properties characteristic of noradrenergic neurons; in adult rats some ciliary neurons have TH-IR and NPY-IR and following neonatal sympathectomy with 6-OHDA both the number of TH-IR cells and the intensity of TH-IR increases. We have counted the number of neurons expressing detectable levels of TH and/or NPY in adult ciliary ganglia. 52% of neurons in control ganglia and 71% of neurons in ganglia from 6-OHDA treated animals contained TH-IR while 25% of control cells and in 45% of 6-OHDA treated animals possessed NPY-IR. TH and NPY were preferentially but not exclusively colocalized. Immunoblots with anti-TH disclosed a 62Kd band in extracts of sympathetic and ciliary ganglia but not liver; this band is twice as intense in ciliary extracts from 6-OHDA treated rats. *In situ* hybridization revealed that both TH and NPY message were present at varying levels in most ciliary neurons. Interestingly, while the message for TH was increased in 6-OHDA rats, reflecting the increase in TH-IR in the ganglion, NPY message was present in the same proportion of cells at the same level in control and 6-OHDA rats. This raises the possibility that posttranscriptional events, either translation or processing, are responsible for the increased peptide. Because nerve growth factor is more available to parasympathetic neurons in sympathectomized animals, it is a candidate for mediating the increase in TH and NPY in 6-OHDA treated rats. Consistent with this possibility, we found that almost all ciliary neurons in control and sympathectomized rats expressed IR for the NGF receptor. Thus, the levels of TH and NPY in the ciliary ganglion are regulated by environmental factors but the level of regulation differs for the two proteins.

481.8

TWO POPULATIONS OF ADULT RAT DRG NEURONS THAT DIFFER IN THEIR READINESS TO EXTEND NEURITES. D.S. Smith and J.H.P Skene. Dept. Neurobiology, Stanford University, Stanford, CA 94305

We are using primary neuronal cultures to investigate the signals for and the intracellular changes involved in the initiation of axonal growth. Cultured neurons from adult rat dorsal root ganglia (DRG) contain two populations of neurons distinguishable by rapid vs. delayed neurite outgrowth. Within 12 hrs after plating, approximately 20% of neurons have extended neurites, and the percentage of process-bearing neurons plateaus at this level for the next ten hours. Beginning at 22 hours the number of neurons with processes increases rapidly, so that by 32 hours 85% of neurons have neurites. The biphasic shape of this growth curve suggests that a small population of adult DRG neurons are in a 'primed' or prepared growth state. Peripheral axotomy performed *in vivo* one week prior to culturing results in a shift from a biphasic to a monophasic growth curve. In these cultures, approximately 70% of neurons possess neurites by 16 hours and 90% by 22 hours, which corresponds to the end of the early plateau observed in control cultures. This finding suggests that normal, mature ganglia contain a population of cells which constitutively exist in a growth state exhibited by the majority of neurons only after axotomy. Previous studies have shown that a subpopulation of uninjured, adult DRG neurons express high levels of the growth-associated protein GAP-43. We are investigating the possibility that normal adult ganglia contain a population of cells that maintain both a capacity for axon growth and a pattern of gene expression usually associated with developing or regenerating neurons.

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481.9

NEURONAL PROPERTIES OF IDENTIFIED CORTICOSPINAL CELLS IN VITRO FOLLOWING SPINAL AXOTOMY IN VIVO. G.-F. Tseng,D. A. Prince. Dept. Neurol. & Neurol. Sci., Stanford Univ., Sch. of Med., Stanford, CA 94305.

We investigated the effects of axotomy on the physiology and morphology of mammalian corticospinal neurons. Control recordings in neocortical slices from nonaxotomized cells, retrogradely labeled with rhodamine beads and double-labeled with biocytin, showed that 3 types of spiking behavior (adapting, regular spiking and regular spiking with depolarizing afterpotentials) were present in the identified corticospinal neurons(Tseng & Prince, Soc.Neurosci.Abstr.,15:660). In the present experiments we examined the physiology and morphology of double-labeled corticospinal cells, 3(n=11), 9(n=13), and 12 months(n=20) following axotomy at the C2-3 level. Although all 3 types of spiking behavior were present, there were significant alterations in several membrane parameters in the axotomized cells. The input resistance, slope of steady-state firing rate versus applied current, steady-state firing rate at a fixed amplitude of current injection, and the membrane time constant were all significantly increased ($p<0.05$). Also, fewer axotomized cells generated a measurable slow AHP, compared to control cells. No qualitative differences in evoked synaptic responses were noticed between control and axotomized neurons. Morphologically, axotomized cells retained their typical pyramidal shape, dendritic domain, axonal arborization and dendritic spines, however quantitative analysis of these variables has not yet been completed.

Our physiological and morphological results confirm the long survival of mammalian corticospinal neurons after distant (spinal) axotomy. These cells retain their general physiological characteristics such as a diversity of spiking behavior. However there are significant alterations in intrinsic membrane properties evidenced by increases in input resistance, membrane time constant, and f/I slope. These changes suggest that remodelling of basic membrane properties occurs following axotomy, which tends to increase neuronal excitability and the response to depolarizing inputs. These alterations may contribute to increases in brain excitability following CNS injury. Supported by NIH grants NS06477 and NS12151 from the NINDS and a Pimley Postdoctoral Fellowship to G.-F. T.

481.10

FURTHER EVIDENCE FOR SYNAPTIC REARRANGEMENTS OF INPUTS TO ONUF'S NUCLEUS (ON) FOLLOWING SPINAL CORD TRANSECTION IN ADULT CATS. J.C. Bresnahan, L.H. Lin*, M.G. Leedy and M.S. Beattie. Depts. of Anatomy and Surgery, and Neuroscience Program, Ohio State Univ., Columbus, OH 43210.

We have previously reported a shift in the size and type of terminals apposed to proximal dendrites and somata of ON motoneurons (MNs) after spinalization. We have extended this EM analysis to distal dendrites (DD) in ON using similar quantitative methodology. DDs located in close proximity to MNs labelled with HRP applied to the pudendal nerve were examined in normal (N), acute (Ac; 4 day) and chronic (Ch; 10-11 wk) spinalized cases (n=4/grp). There were fewer terminal appositions per DD and fewer terminals per unit of DD membrane in the Ac as compared to the N group, with some recovery in the Ch. A reversed pattern was observed for glial apposition (Ac>Ch>N). Terminals apposed to DD were smaller in both the Ac and Ch as compared to Ns, and there was a greater proportion of terminals containing round synaptic vesicles in the Ac and Ch groups vs. Ns. These results indicate similar synaptic rearrangements on DD of ON neurons as were observed on the proximal regions of ON MNs. (Supported by NS-10165)

481.11

WILL PRIOR SPINAL INJURY ENHANCE RECOVERY OF HINDLIMB MOTOR FUNCTION AFTER SPINAL TRANSECTION IN ADULT CATS? S.P. Lahr, and M.E. Goldberger, Med. Col. of Pennsylvania, Philadelphia, PA 19129.

The present studies were designed to determine if recovery from a previous spinal cord hemisection will enhance recovery of hindlimb locomotion in adult cats after a subsequent spinal transection. Previous studies have shown a reduction of spinal segmental reflex depression (spinal shock) with this paradigm (Chambers et al., 1966). Preoperatively, adult cats were trained to perform bipedal treadmill locomotion and monopedal hopping (at 0.2 - 0.6 m/s). One group (n=3) received a T13 spinal transection and a second group (n=4) received a right spinal hemisection at the T13 level followed 1-2 months later by a spinal transection one segment rostrally. Locomotion was assessed quantitatively using a motion analysis system. In animals with only spinal transection, recovery of air stepping, monopedal hopping, and bipedal stepping is not seen until the end of the second week p.o. at the earliest. In contrast, in animals with spinal hemisection + transection, recovery is seen during the first week p.o. following the spinal transection, and begins on the chronic side (ipsilateral to the hemisection) as early as the second day p.o. Although both hindlimbs exhibit precocious recovery, there is also a temporary asymmetry in motor function between the two hindlimbs. The time course of recovery after transection was also related inv ersely to the magnitude of the deficit seen after the preceding hemisection. We conclude that spinal shock interferes with recovery of motor function since reducing spinal shock enhances hindlimb motor recovery following spinal transection and that the reorganization that occurs after the first lesion contributes to recovery after the second. (Supported by the American Paralysis Foundation and NIH Grant NS24707).

481.12

USE-DEPENDENT MODIFICATION OF Ia AFFERENT SYNAPTIC STRENGTH. C.B. Webb and T.C. Cope Physiology and Biophysics, Hahnemann Univ., Phila. PA 19102

The silencing of a large fraction of afferent input to the lumbosacral cord leads to enhanced synaptic strength of the Ia afferent to alpha-motoneuron connection (Manabe et al., J. Neurosci. 1989). The present study employs EMG confirmation of osmotic mini-pump delivery of TTX through a nerve cuff around the cat MG nerve to examine specific disuse-induced changes in synaptic efficacy independent of changes in the remaining hindlimb afferent input. In cats whose MG nerve had been silenced for two weeks, the mean aggregate Ia EPSP amplitude was 3.1mV ± 2.1(SD), significantly different (unpaired t-test, $p<.01$) from that recorded in control animals, 2.2mV ± 1.7(SD). Rise time, half-width, rheobase, AHP, and Vm for the two samples were not significantly different. The ratio of LG/MG EPSP amplitude in LG motoneurons (greater than one in controls) was less than one in 20% of the cases measured following TTX treatment. The present study confirms that the silencing of afferent input leads to an increase in aggregate Ia EPSP amplitude, and further, shows that the disuse of a specific set of synaptic connections leads to an increase in the strength of those connections. This lab is currently using spike-triggered averaging to measure connectivity and EPSP amplitudes of single Ia afferents following disuse to address possible mechanisms, e.g. increase in functional contacts, or increased transmitter release. (Supported by NIH NS21023)

481.13

EXPRESSION OF CHOLINE ACETYLTRANSFERASE (ChAT) AND NERVE GROWTH FACTOR RECEPTOR (NGFr) WITHIN HYPOGLOSSAL MOTONEURONS FOLLOWING NERVE INJURY. D. Armstrong, R. Brady, R. Hayes, and R.G. Wiley. FGIN, Georgetown University, Washington, D.C. 20007.

In the present study we employed light microscopic immunocytochemistry in order to investigate the temporal response of ChAT and NGFr within hypoglossal motoneurons following unilateral transection or crushing of the XII nerve. In control rats (i.e., sham operated) virtually all the motoneurons of the XII nucleus were darkly labeled for ChAT and devoid of NGFr immunoreactivity. Transection (ChAT): Three days following nerve transection the intensity and the number of ChAT-positive neurons were markedly reduced on the axotomized side compared to the non-lesioned side. This decrease in labeling continued until nine days when virtually no ChAT-labeled cells were present on the lesioned side. In contrast, no loss of hypoglossal neurons were found using Nissl stains. Importantly, the persistence of these motoneurons suggests that the absence of ChAT is not an absolute indicator of cell death. This absence of ChAT immunolabeling persisted for several days, yet by 30 days many motoneurons begin to re-express the enzyme. Transection (NGFr): As early as one day following the lesion motoneurons begin to express NGFr immunoreactivity. This response was very robust three days following the lesion and continued throughout all time points thus far examined. Importantly, NGFr immunoreactivity could be seen at times after many motoneurons began to re-express ChAT. Crush (ChAT): Crushing of the hypoglossal nerve resulted in a transient reduction in the overall intensity of ChAT immunoreactivity yet produced little if any loss in the number ChAT neurons. The decrease labeling was most apparent 3 to 12 days following the lesion. Crush (NGFr): Nerve crush also resulted in the transient expression of NGFr immunoreactivity which was most robust 3 to 9 days following the lesion. At present the mechanism underlying the lesion induced expression of NGFr is under study.

481.14

IMMUNOHISTOCHEMISTRY OF HYPOGLOSSAL AFFERENTS AND EFFECTS OF AXOTOMY. R.G. Wiley, K. Crews* & C. Hunt*. Experimental Neurology, DVAMC, Nashville, TN 37212 and Vanderbilt University.

In the present study, we sought to determine which neurotransmitters can be demonstrated in the nucleus using immunohistochemical techniques and the effects of hypoglossal nerve transection on those inputs. Adult Sprague-Dawley rats of both sexes were anesthetized and underwent unilateral hypoglossal transection with suture ligation of the proximal stump. After 3-66 d, rats were perfused with aldehyde fixative and frozen sections of the medullas processed for indirect peroxidase immunohistochemistry. Leu-enkephalin and glutamate gave moderate terminal labelling in the hypoglossal nucleus. Substance P, NPY, Met-enkephalin, CRF, PNMT and beta-endorphin gave light terminal labelling. DBH, VIP, CCK, neurotensin and somatostatin did not stain the nucleus. Glutamate staining was decreased in the hypoglossal nucleus ipsilateral to chronic axotomy. These results suggest several transmitter systems for studies of the reaction to axotomy or loss of motor neurons. (Supported by DVAMC.)

481.15

c-FOS AND SILVER DEGENERATION REACTIONS IN ADULT RAT BRAIN STEM FOLLOWING ACUTE AND CHRONIC DENTAL INFLAMMATION. M.R. Byers, L.E. Westrum, W.K. Dong, M.J. Iadarola. Anesthesiol., Biol. Structure, Neurol. Surgery; Univ. of Washington, Seattle, WA 98195; Neurobiol. & Anesth., NIDR, NIH, Bethesda, MD 20892.

We have used immunocytochemistry to analyze the expression of c-Fos and Fos related antigens in the brain stem of the rat adult at 5-6 hr after pulp exposure injury to the right maxillary first and second molars. The acute group was compared to others with the same injury that survived 5-6 days or 15 days. Postoperative behavior and weight gain were normal after this surgery. After fixation alternate transverse sections were processed for Fink-Heimer silver stain or immunocytochemistry. Three different response patterns were found. Acute Fos/Fra response included many ipsilateral caudalis neurons, fewer contralateral caudalis neurons, plus many bilateral stained neurons in the periobex VL reticular zone, lamina X, medial and lateral solitary nuclei and area postrema. Chronic Fos/Fra response only occurred in caudalis neurons and a cluster of neurons in the rostral lateral solitary nucleus at the level of interpolaris, both with a predominantly ipsilateral response. Silver Degeneration was not prominent in the Fos reactive sites but was located more rostrally, especially in interpolaris/oralis. These distinctive responses to dental inflammation in the rat offer a useful model for analysis of central plasticity in response to peripheral inflammation. Supported by NIH grants DE05159 and DE04942.

481.16

CAPILLARY GROWTH IN CEREBELLAR CORTEX OF ADULT RATS AFTER EXTENSIVE PHYSICAL EXERCISE. A.A. Alcantara, J.E. Black, K.R. Isaacs, B.J. Anderson, & W.T. Greenough. Beckman Inst, Depts Psychol and Cell & Struct Biol, Neuroscience Prog, & Coll Med., Univ Illinois, Urbana, IL 61801 USA.

Previous work has demonstrated new capillary formation in visual cortex of rats in association with synaptogenesis and tissue volume expansion (e.g., Black, et al, Neurosci Lett, 83:351, 1987; Black, et al, Neurobiol Aging, 10:353, 1989). Angiogenesis might also occur when neuropil metabolism is chronically elevated, such as repetitive activation of neurons and synapses.

We examined microvasculature in the paramedian lobule (PML) of the cerebellum of 38 adult female rats put in 4 experimental groups for 30 days. Acrobatic Condition (AC) rats were given extensive visuomotor training on difficult tasks, e.g. traversing rope ladders and thin dowels. This group tested effects of learning on synaptic connectivity. Forced eXercise (FX) rats ran on a treadmill, and those in the Voluntary eXercise condition (VX) had free access to a running wheel. Inactive Condition (IC) rats did not have access to either learning or exercise. Semithin sections of PML were drawn using a camera lucida, and the area density of blood vessels in the molecular layer was determined. Previous work demonstrated substantial synaptogenesis and volume expansion in the AC rats and none in the other groups. The significant increase in vessel density in the FX and VX groups without changes in neuropil volume indicates infiltration of this tissue by new blood vessels. The PML is activated by limb movements, and presumably the increased metabolic demand in the exercise groups elicited compensatory angiogenesis.

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481.17

5'NUCLEOTIDASE (5'N): A NEW CYTOCHEMICAL MARKER OF PLASTIC STATUS OF SYNAPSES IN THE CEREBELLAR CORTEX OF X-IRRADIATED RATS AND REELER MICE. Y. Bailly*, S.W. Schoen*, N. Delhaye-Bouchaud*, G.W. Kreutzberg and J. Mariani. Inst.Neurosc. Univ. P.& M. Curie, Paris, France and Max-Planck Inst., Martinsried, FRG.

We reported recently that a single postnatal (P5/P6) X-irradiation of the rat cerebellum impedes the elimination of redundant synapses between climbing fibers (CFs) and Purkinje cells (PCs) that occurs during normal development. A similar polyneuronal innervation is also maintained on ectopic PCs in the reeler mouse cerebellum. Cytochemical detection of 5'N, an adenosine producing ecto-enzyme, revealed its transient appearance on the synapses made by the climbing, mossy (MF) and parallel fibers (PF) in normal developing rats. Interestingly,the elimination of CF synapses and 5'N labeling occur within the same days. Using identical cytochemical methods, we now report at the electron microscopical level a conspicuous 5'N activity in most of the CF synapses in the cerebellum of adult X-irradiated rats and reeler mice. In the X-irradiated rats, 5'N activity is also maintained in many MF and some PF synapses. These results suggest that 5'N is a functional cue to characterize the maturation of the excitatory synapses in the rat cerebellar cortex. In the CF synapses it could herald an immature plastic status that is maintained until adulthood in the synapses of the cerebella studied.

482.1

BIOCHEMICAL COMPARISON OF ASTROCYTE MATURATION *IN VITRO* AND *IN VITRO*. George M. Smith[1], James Jacobberger[2], and Robert H. Miller[2]. [1]Dept. of Neurosurgery, Baylor College of Medicine, 6560 Fannin, Houston, Texas 77030; [2]Dept of Neurosciences, Case Western Reserve Univ., Cleveland, Ohio 44106.

The ability of astrocytes to support axon outgrowth diminishes during maturation of the central nervous system. This reduction also occurs as cultured astrocytes aged *in vitro*. Several molecules, including NCAM and laminin have been demonstrated by antibody inhibition studies to be involved in astrocyte mediated axon outgrowth. In this study, the expression of HNK-1, NCAM, laminin, and GFAP was compared during *in vivo* and *in vitro* astrocyte maturation by indirect immunofluorescence, and analyzed by flow cytometry. In parallel experiments, tissue sections of maturing rat brain were similarly labeled and examined. The results indicted that at birth the majority of cortical astrocytes expressed high levels of the three cell surface molecules. While number of astrocytes expressing HNK-1 and NCAM diminished, both *in vivo* and *in vitro*, during astrocyte maturation, there was no significant decrease in the number of astrocytes expressing laminin. To distinguish between differentiation of a single astrocyte population or differential replication of multiple astrocyte populations, *in vitro* samples were labeled for NCAM or HNK-1, counter stained for DNA content, and analyzed by flow cytometry. Cell cycle phase fraction analysis indicated no significant difference in replication rated between those astrocytes that did and did not express HNK-1 and NCAM. Therefore, the reduction in HNK-1 and NCAM expression correlates with the reduction in axon outgrowth promoting properties of astrocytes. Furthermore, using the above markers, astrocyte maturation appears temporally and biochemically indistinguishable *in vivo* and *in vitro*.

482.2

FGF and PDGF Differentially Regulate the Developmental Course of Oligodendrocyte Progenitors Placed in Culture. A.L. Gard and S.E. Pfeiffer. Department of Microbiology, University of Connecticut School of Medicine, Farmington, CT 06032.

Three consecutive stages of the oligodendrocyte (OL) lineage are distinguished immunocytochemically in rodent brain and optic nerve by the ordered appearance of cell surface antigens, A2B5, O4 and galactocerebroside (GalC). Recently we demonstrated that OL progenitors bearing the $A2B5^+O4^+GalC^-$ phenotype, termed "proligodendrocytes," represent a distinct lineage interval *in vivo*. When placed in culture these cells differentiate in a chemically defined medium to sequentially express GalC and the major myelin proteins (Gard and Pfeiffer, Development 106: 119, 1989). To examine whether this stage is targeted by mitogens implicated in OL development, proligodendrocytes purified directly from postnatal telencephalon were cultured under defined conditions with either basic fibroblast growth factor (FGF) or platelet-derived growth factor (PDGF) added at the time of seeding. At physiologically relevant concentrations, FGF acted as a potent mitogen for multipolar proligodendrocytes (ED_{50} = 1 ng ml^{-1}), producing a maximal 5-fold increase in bromodeoxyuridine labeling index (20% in 6 hrs) compared to control cultures (4%) at plateau levels (2-5 ng ml^{-1}), without altering the timecourse of differentiation into $GalC^+$ OL (20% by 1 DIC, 80% at 2 DIC). In contrast, PDGF caused ~70% of the proligodendrocytes to transiently revert by 1 DIC to cells resembling their precursors (ED_{50} = 3 ng ml^{-1}), characterized by an $A2B5^+O4^-$ phenotype and bipolar morphology. Reverted cells proliferated, re-expressed O4 on their surface after 1-2 days as bipolar cells, and finally resumed differentiation on a delayed schedule. The reversion was verified by single cell analysis, and also apparent in cultures of dissociated 7-day optic nerve cells of which proligodendrocytes comprised the predominant lineage stage at the time of seeding. The results suggest that exogenously administered PDGF rapidly resets the differentiation program of cultured proligodendrocytes born *in vivo*, which otherwise utilize FGF as their mitogenic signal. Supported by NIH Grant NS10861.

482.3

MHC ANTIGEN EXPRESSION IN THE RAT RETINA FOLLOWING INTRACRANIAL OPTIC NERVE SECTION. K. Rao,J.D.Radel and R.D.Lund. Department of Neurobiology Anatomy and Cell Science, University of Pittsburgh, School of Medicine, Pittsburgh, PA 15261

Previous studies (Streit,W.J.,et al., Exp. Neurol.,105:115,1989) have shown that axotomy of motorneurons causes expression of both class I and class II MHC antigens on microglia around cell bodies. We have examined here, MHC antigen expression patterns in the adult rat retina after intracranial optic nerve section, using the opposite (unlesioned) eye as a control. In this system, regeneration does not normally follow axotomy and the microglia are more broadly distributed throughout the retina. The animals are fixed at regular intervals following optic nerve section and cryostat sections are stained with antibodies against microglia and MHC antigens. Within 3 days of axotomy, scattered microglia within the inner plexiform layer express class I MHC antigens. By 11 days the majority of expressing cells are closely associated with the optic fiber layer. Although cells in the choroid in the normal and experimental retina express class II antigens, no staining of class II-positive cells was seen in any of the retinal layers.

This suggests that under conditions of retrograde degeneration, like those in orthograde degeneration (Smetanka,et al., Brain Res.,in press), expression of MHC class I antigens can be present without class II expression. It also indicates that expression patterns associated with retrograde degeneration vary depending on the system studied.

Supported by The Winters Foundation.

482.4

ASTROCYTES RELEASE A NITROSYL COMPOUND WITH VASORELAXANT PROPERTIES. S. Murphy and G. Welk*. Dept. of Pharmacology, College of Medicine, Univ. of Iowa, Iowa City, IA 52242.

Astrocytes release a variety of vasoactive eicosanoids. In addition, these cells release a non-prostanoid, labile vasodilator in response to bradykinin (Murphy et al., J. Neurochem. 54, 1990) which we have termed astrocyte-derived relaxing factor (ADRF). This factor is nitric oxide (NO) or a nitrosyl compound derived from arginine. To investigate further the regulation of ADRF release, primary astrocyte cultures were exposed to a variety of agents which interact with surface receptors or with intracellular effectors, and NO release determined by a chemiluminescence technique.

Quisqualate and norepinephrine, but not a range of other receptor agonists, were effective in evoking NO release from astrocytes. The noradrenergic effect was not inhibited by ß-receptor antagonists, was not mimicked by clonidine or blocked by yohimbine, but was reversed by the α_1 antagonist prazosin. AMPA did not mimick the effect of quisqualate, suggesting involvement of the metabotropic excitatory amino acid receptor. Both A23187 and thapsigargin, a non-phorbol tumor promoter which selectively discharges intracellular calcium, also evoked NO release. Thus, astrocytes, like endothelial cells and neurons, release a NO-containing compound which could potentially interact with soluble guanylate cyclase in adjacent cells, and so act as an intercellular signal.

482.5

ACTIVATORS OF PHOSPHOLIPASE D IN ASTROCYTES G. Bruner and S. Murphy Dept. of Pharmacology, College of Medicine, Univ. of Iowa, Iowa City, IA 52246

Astrocytes synthesize and release eicosanoids upon stimulation with purinergic agonists and agents such as calcium ionophore and phorbol esters. The precise mechanism by which this process occurs is unknown, but could involve phospholipase D (PLD). To determine the mechanism of PLD activation, and learn more about its potential role in eicosanoid production, primary astrocyte cultures were prelabeled with [^{3}H] myristate, and the formation of phosphatidylethanol (PEt) was determined in the presence of ethanol after stimulation with various agents for 30 min. Formation of PEt by phorbol myristate acetate (PMA; EC_{50}=6nM) was abolished by prior down-regulation of protein kinase C (PKC). Receptor agonists which might be predicted to stimulate PLD through activation of PKC include ATP, bradykinin, glutamate, and carbachol; each of these agents failed to stimulate formation of PEt in astrocyte cultures. Thapsigargin (Tg), a novel compound which has been reported to selectively discharge intracellular calcium stores but not activate PKC, mobilized arachidonic acid and evoked thromboxane A_2 production in a dose dependent manner (EC_{50}=12nM). Tg also stimulated PEt formation, however the effects of Tg on PLD were abolished when PKC was first down-regulated. It is apparent that PLD is not coupled to surface receptors in astrocytes, including purinergic receptors which are known to be linked to eicosanoid production. Physiological regulators and the role of PLD in astrocytes have yet to be defined.

482.6

SV40 T ANTIGEN MEDIATED IMMORTALIZATION PRESERVES THE NEURITE OUTGROWTH PROMOTING PHENOTYPE OF CNS ASTROCYTES. P.S. Frisa*, M.N. Goodman, G.M. Smith, J. Silver and J. Jacobberger. Case Western Reserve University, Depts. of Genetics and Neurosciences, Cleveland, OH 44106

CNS maturation is accompanied by an attenuated neural regeneration capacity that is correlated with a change in the neurite outgrowth promoting properties of astrocytes. Similarly, the outgrowth phenotype of astrocytes in vitro is dependent on the age of the animal at the time of astrocyte isolation as well as length of time in culture. In general, immortalization by single dominant oncogenes correlates with inhibition of cellular differentiation. Thus, immortalization of astrocytes at timed intervals from the maturing brain should yield cell lines trapped in differentiated states representative of the maturing CNS. This hypothesis has been tested by transducing rat and mouse cortical astrocyte cultures with SV40 large T antigen.

Morphologically, the resulting clonal cell lines are type 1 astrocytes. Many express GFAP and T antigen, are contact inhibited, and capable of entering a quiescent cell cycle phase. These cell lines have been tested for the ability to promote neurite outgrowth using embryonic chick retinal ganglion cells. Clonal lines derived from aged primary mouse astrocyte cultures expressed a range of abilities to promote neurite outgrowth, however, the mean value for all mature lines tested was equivalent to primary aged cells. Two cell lines derived from primary rat 2 day cultures both supported neurite outgrowth at the same level as primary 2 day cultures.

Thus, it has been demonstrated that SV40 T-immortalized astrocytes are phenotypically similar to the differentiating astrocyte population extant in the maturing CNS at the time of isolation and culture, that the outgrowth phenotype is stable, and that the cells are relatively non-tumorigenic in transformation phenotype.

482.7

NEUROTRANSMITTER REGULATION OF GLYCOGEN METABOLISM IN CULTURED ASTROGLIA. O. Sorg*, D. L. Feinstein and P.J. Magistretti. Institut de Physiologie, Faculté de Médecine, Université de Lausanne, CH-1005 Lausanne, Switzerland.

In recent years astrocytes have been shown to possess receptors for various neurotransmitters.These observations further support the notion of neurotransmitter-mediated functional interactions between neurons and astrocytes.We have previously demonstrated that NA, VIP, adenosine (all via cAMP) and K^+ (via Ca^{+2}) promote the hydrolysis of ^{3}H-glycogen newly synthesized from ^{3}H-glucose in mouse cerebral cortical slices. Since, in vivo, glycogen is predominantly localized to astrocytes, we screened these and other neuroactive agents for their potential glycogenolytic effect in primary cultures of astrocytes prepared from neonatal mice. Cultures were grown for 2 to 3 weeks in DMEM containing 10% FCS and 25 mM glucose. On the day of experiment serum was removed and glucose levels decreased to 5 mM; after 4 hrs, substances under scrutiny were added for 30 min. Endogenous glycogen was measured by the hexokinase/glucose-6-phosphate dehydrogenase fluorometric procedure following amyloglycosidase treatment of the samples. In agreement with our observations in slices and with early experiments in cultured astrocytes in which glycogen had been pre-labelled with ^{3}H-glucose, NA (100µM) and VIP (1 µM) hydrolyzed ≈ 70 % of endogenous glycogen; other active agents included adenosine, isoproterenol, dopamine, dbcAMP and the phorbol ester PDBu, all at 100 µM. In contrast to slices, K^+ at 25 mM was inactive; however in the presence of the Ca-channel agonist BAY-K 8644, K^+ elicited ≈ 40% glycogen hydrolysis. A glycogenolytic response to K^+ (≈30%) was also observed in cultures treated with 1 mM dbcAMP for 3 days prior and up to 5 hrs before the experiment. This treatment also results in increased (≈4-fold) glycogen levels and in a decreased response to NA, VIP and adenosine.The mechanism(s) of this "desensitization" is currently being investigated.

482.8

VIP RECEPTORS AND VIP-STIMULATED cAMP FORMATION IN CULTURED ASTROGLIA AND CEREBRAL MICROVESSELS. D.L. Feinstein, C. Rossier*, N. Yu*, J.-L. Martin, P.J. Magistretti. Institut de Physiologie, Faculté de Médicine, Université de Lausanne, CH-1005, Lausanne, Switzerland.

In mouse neocortical slices, VIP interacts synergistically with noradrenaline (NA), adenosine and phorbol esters to increase cAMP levels. However, it is not known which cell type(s) are the target(s) for these interactions. We have therefore begun a series of studies in cultured astroglia and acutely isolated microvessels to determine if in either of these preparations a synergistic response occurs. We first characterized VIP binding to cultured astroglia. Specific binding of ^{125}I VIP was rapid, saturable, and reversible, and revealed the presence of 2 types of binding sites, a high affinity site with a Kd of 1 nM and Bmax of 150 fmoles/mg protein, and a low affinity site with a Kd of 85 nM and Bmax of 1500 fmoles/mg protein. The related peptide PHI inhibited VIP binding with an IC_{50} of 10 nM, while secretin at up to 1 µM had no effect on VIP binding. The presence of 0.1 µM VIP for 30 minutes elicited an increase in cAMP levels (4-8 fold over basal levels). Increases were also observed with NA (10 µM) and adenosine (7.5 µM), but not with the phorbol ester PDBu. Preliminary experiments indicate that all 3 of these molecules interact synergistically with VIP. Serum deprivation for 4 days caused loss of the synergism with NA and adenosine, while the interaction with PDBu was maintained. In acutely isolated mouse cerebral microvessels, both NA and VIP elicited increases in cAMP (EC_{50} of 500 nM and 50 nM, respectively). However, no synergism was found as the response to the combination of these drugs was the sum of the individual responses.

482.9

INHIBITORS OF GLYCOPROTEIN PROCESSING GLUCOSIDASES BLOCK OLIGODENDROCYTE DIFFERENTIATION. N.R. Bhat. Department of Biochemistry and Sanders-Brown Center on Aging, University of Kentucky, College of Medicine, Lexington, KY 40536.

Previous studies (Bhat, N.R., J Neurosci Res. 20, 158, 1988) have shown that inhibitors of processing glucosidases reversibly inhibit oligodendrocyte (OL)-specific activities in heterogeneous cultures of developing rat brain cells. The present study examines the direct effect of processing inhibitors on the proliferation and differentiation of isolated OL progenitor cells. The OL progenitor cells isolated from mixed glial cultures of newborn rat brain undergo an initial period of active proliferation (peaking 2-3 day post-plating) followed immediately by a marked induction of differentiated properties of OL, i.e, sulfogalactolipid synthesis and 2', 3'-cyclic nucleotide 3'-phosphohydrolase activity. Castanospermine (cast), a potent inhibitor of processing glucosidases inhibited the induction of these activities without affecting cell proliferation. The effect was concentration-dependent with a maximal 60-80% inhibition observed at 25µg/ml cast. Swainsonine, an inhibitor of processing mannosidases, had no effect on OL differentiation even though it, like cast, prevented the formation of complex-type oligosaccharides. It is concluded that core glycosylation and initial processing of oligosaccharides may be critical for the functioning of specific glycoproteins essential to OL differentiation. (Supported by NMSS grant # RG1927).

482.10

HOMOGRAFTS OF CULTURED FETAL SPINAL CORD ASTROCYTES INCREASE THE SEVERITY OF LESION-INDUCED HIND LIMB DEFICITS J.J. Bernstein, L.A. Gillespie*, P. S. Wilson* and W.J. Goldberg. CNS Injury & Regen., V.A. Med. Ctr., Wash., DC; Depts. Neurosurg. & Physiol., Geo. Wash. Univ. Sch. Med., Wash., DC.

We have previously demonstrated that grafts of unoriented pieces of E14 fetal spinal cord into C3 fasciculus gracilis (C3FG) aspiration pockets ameliorate the deterioration of hindlimb performance. We have now tested the effects of cultured astrocyte grafts, placed in the same aspiration site, on hindlimb performance. Fourteen, 250g Sprague-Dawley rats were placed on a 23-h off and 1-h on water schedule and trained to traverse a 2'x4" horizontal ladder (1/8" bars, 1" apart) for a water reward. Criterion was established as 10 complete ladder traverses on 2 consecutive days. The numbers of hindlimb slips were determined from video tapes. In a "blind" experimental design, trained animals were coded and laminectomy performed at C3. The C3FG was bilaterally sectioned at the rostral and caudal borders of the segment and then aspirated. Seven subjects were chosen at random to receive grafts of 10^5 cultured E18 rat astrocytes. Lesion-only and grafted subjects were tested at 14, 30 and 45 days. Prelesioned animals had 3 hindlimb slips in 20 traverses. At 14 days, lesion-only animals had 10 slips whereas grafted animals had 40 slips. Slips remained at these levels to 45 days. Astrocyte grafts placed in the lesion pocket resulted in decreased rather than increased hindlimb performance as was previously demonstrated using unoriented whole spinal cord grafts. Support: Veterans Affairs

482.11

CYSTEAMINE INDUCES THE 'GOMORI' PHENOTYPE IN CULTURED ASTROGLIA. H.M. Schipper, D.E. Scarborough*, R.M. Lechan and S. Reichlin. Dept. of Neurology, Jewish General Hosp., McGill Univ., Montreal, Canada H3T 1E2 and Dept. Endocrin., New England Med. Center, Tufts, Univ., Boston, MA 02111

Gomori-positive astrocytes have been identified in the periventricular brain in situ and in diencephalic explants on the basis of their endogenous peroxidase activity, affinity for chrom alum hematoxylin, and orange-red autofluorescence. In dissociated fetal rat brain cultures, astrocytes containing cytoplasmic inclusions with the above tinctorial and fluorescent properties represented less than 1% of GFAP-positive astrocytes at day 10 in vitro (DIV). There was a marked increase in the fraction of Gomori-positive astrocytes and their granule content between 10 and 46 DIV. Cysteamine and cystamine, but not ethanolamine or L-cysteine, induced a massive accumulation of Gomori-positive astrocytes when administered from DIV 6-18. As in situ, the peroxidase activity induced by cysteamine appeared to be non-enzyme-mediated insofar as it catalyzed diaminobenzidine oxidation over a wide range of pH (3-11) and could not be inhibited by tissue pre-heating or the catalase inhibitor, aminotriazole. Metallo-porphyrins probably mediate both the pseudoperoxidase activity and autofluorescence in these cells. Alterations of the redox microenvironment or induction of porphyrin/heme biosynthetic enzymes may be the mechanisms responsible for this cyst(e)amine effect.

482.12

INTRACELLULAR pH REGULATION IN CULTURED ASTROCYTES: A MICROELECTRODE STUDY. W. Walz and W. Wuttke*. Dept. of Physiology, Univ. of Saskatchewan, Saskatoon, S7N 0W0, Canada.

Transmembrane transport processes involved in regulating intracellular pH in astrocytes were studied. Primary cultures of cortical astrocytes from newborn Swiss mice were impaled with two-channel pH-sensitive microelectrodes. In bicarbonate-buffered saline pH_i was 7.05 and in HEPES-buffered saline 6.68. In both solutions H^+ was not in electrochemical equilibrium; pH_i was 0.7-1 pH unit more alkaline than expected from passive H^+ distribution. The regulatory mechanisms were studied in bicarbonate-free HEPES-buffered saline. The cells were acidified by applying NH_4^+ for 20-60 sec and the subsequent regulation of pH_i back to normal baseline values was investigated. The mean rate of pH_i recovery was 0.2 pH units min^{-1} which was not changed by removal of external Na^+ or by amiloride (1 mM) exposure. Thus, the cells recovered from an acid load independently of Na^+-H^+ exchange, Na^+-HCO_3^- cotransport or any other bicarbonate- or Na^+-dependent mechanism. Cultured astrocytes have a very high lactate prodution and release rate. During normal conditions the lactate : H^+ ratio is higher than 1.0. We suggest that lactate-H^+ cotransport via passive diffusion and the monocarboxylic acid carrier is responsible for the extrusion of the acid load.

482.13

NEURITE OUTGROWTH PROMOTION BY RAT OLFACTORY BULB CULTURES AND CELL LINES. M. N. Goodman[1], J. Silver[2] and J. W. Jacobberger[1]. Depts. of Genetics[1] and Neurosciences[2], Case Western Reserve University, Cleveland, OH 44106.

The rat olfactory bulb (OB), an area of the CNS re-innervated throughout life, contains two glial cell types within the outer lamina and glial limitans. Bulb astrocytes are morphologically and antigenically like type 1 astrocytes while the ensheathing cells of the bulb are like non-myelinating Schwann cells. Both cell types are intermingled, but sensory axons associate preferentially with ensheathing cells. When the adult OB is lesioned, bulb astrocytes at the lesion site form a glial scar, however, re-innervation occurs by growth of axons around the scar. To investigate the role of each cell type in adult bulb re-innervation, the neurite outgrowth promoting ability of immortalized adult OB cell lines, neonatal and adult OB glial cultures, cerebral cortex (CC) astrocyte cell lines, and CC astrocyte cultures were measured and compared.

Neonatal OB and CC cultures promoted outgrowth equivalently. Adult OB cultures promoted outgrowth at an intermediate level between neonatal cultures (the highest observed levels) and adult CC cultures (the lowest levels). Adult ensheathing cell lines promoted higher levels of outgrowth than adult astrocyte lines derived from either OB or CC, and were equivalent to or greater than OB and CC neonatal cultures. Neurite outgrowth over adult OB and CC astrocyte lines was equivalent to or lower than that over adult OB cultures.

These results suggest that ensheathing cells retain a high level of axon growth-promoting capacity relative to OB astrocytes, and that the neurite outgrowth capacity of OB astrocytes, like CC astrocytes, is reduced during maturation. Purification of ensheathing cells and astrocytes from OB cultures for neurite outgrowth studies is in progress.

482.14

THE VARIABLE MITOTIC RESPONSE OF ADULT RAT OLIGODENDROCYTES RS Vick' and GH DeVries Dept Biochemistry, Medical College of Virginia, Richmond, VA 23298

Adult oligodendrocytes (OLGs), isolated and cultured according to Vick, et al. (J Neurosci Res 25:524-534, 1990), were stimulated with pituitary extract (PX) or co-cultured with dorsal root ganglion (DRG) neurites for various times up to 6 d. ^{3}H thymidine was added for the final 48 h in the DRG co-culture experiments. Previous studies demonstrated that adult OLGs are virtually unresponsive to most soluble and particulate mitogens (Vick and DeVries, J Neurosci Res, submitted 1990). Fluorescence activated cell sorting (FACS) demonstrated that 100 % of the unstimulated cultured OLGs were in a quiescent (G_0/G_1) state. Autoradiography demonstrated that 20 % of the PX stimulated OLGs proliferated after 3 d; FACS analysis of the OLGs demonstrated that this proliferation was not synchronized. Co-culture of OLGs for 2, 3, and 4 d yielded a labeling index (LI) of 7 %. By 5 d the LI increased to 25 % and by 6 d 40 % of the cells were labeled. The variability in the lag of the mitogenic response of the adult OLGs indicates that they are in variable states of responsiveness to mitogens. Thus the morphologically and immunologically homogeneous population of adult OLGs respond to mitogens in a heterogeneous manner. (Supported by NIH NS07288 and MS Society 5073)

482.15

INTERLEUKIN-1 AND TUMOR NECROSIS FACTOR INCREASE [3H]Ro5-4864 BINDING IN TYPE-1 ASTROCYTES IN CULTURE. Y.J. Oh, G.J. Markelonis, T.H. Oh, and J.W. Francis. Dept. Anat., Univ. Maryland Sch. Medicine, Baltimore, MD 21201.

Injuries to CNS tissue induce a number of biochemical changes within astrocytes including the up-regulation of glial fibrillary acidic protein (GFAP) and peripheral benzodiazepine (PBZD) receptors. Since our laboratory has been investigating whether particular cytokines influence these changes, we determined the effects of physiological levels of both interleukin (IL)-1 and tumor necrosis factor (TNF) on PBZD receptors using [3H]Ro5-4864 as a ligand. Both type-1 and type-2 astrocytes were prepared from neonatal rat brain. Specific binding of 2 nM ligand to type-1 astrocytes reached equilibrium between 30 to 60 min and was half maximal at 10 min. Surprisingly, [3H]Ro5-4864 binding to type-2 astrocytes was barely above the level of non-specific binding and measured only 0.5% of the binding observed in cultures of type-1 astrocytes. After treating cultures with IL-1 beta for 5 days, the binding of [3H]Ro5-4864 to type-1 astrocytes doubled, whereas no changes were seen within type-2 astrocytes. Both IL-1 alpha and TNF were shown to increase the binding of [3H]Ro5-4864 in a dose-dependent manner. Regardless of treatment, specific binding to type-2 astrocytes was less than 0.5% of the binding to type-1 astrocytes. These data suggest that (i) PBZD receptors are present only in type-1 astrocytes, (ii) specific binding of [3H]Ro5-4864 can be increased by IL-1 and TNF in a dose-dependent manner, and (iii) cytokines may play a role in the "activation" of astrocytes following injury to the brain. Supported in part by NIH grant (NS 15013) and the Bressler Research Fund.

482.16

DYSMYELINATION IN BOVINE ß-MANNOSIDOSIS: WHITE MATTER LESIONS IN A LYSOSOMAL STORAGE DISEASE IN SALERS CALVES. K.L. Lovell, M.Z. Jones, and B. Abbitt*. Dept. Pathology, Mich. State Univ., E. Lansing, MI 48824 and Texas Veterinary Medical Diag. Lab., College Station, TX 77841

An inherited defect in lysosomal ß-mannosidase activity has been previously reported in goats and humans. Affected goats are unable to rise and show neurological deficits, including intention tremor, while most of the human cases have shown mental retardation and deafness. Pathological changes in affected goats include cytoplasmic vacuolation and myelin deficits, with consistent regional variation. In this study of bovine ß-mannosidosis, the glial cell and myelin abnormalities were investigated in Salers calves recently identified as affected with ß-mannosidosis. Brain and spinal cord tissues from three affected animals and one control animal were examined. Paraffin-embedded sections were stained with hematoxylin & eosin and with luxol fast blue-periodic acid Schiff-Holmes stains. Semi-thin Epon-embedded sections were stained with toluidine blue. In the cerebral hemispheres, there was a substantial decrease in the volume of white matter in the affected calves. Myelin deficiency was apparent throughout the brain, with marked variation in severity among regions. For example the myelin deficit was more severe in the corpus callosum, where practically no myelin sheaths were present, than in the cerebellar white matter. The spinal cord showed much greater amounts of myelin than brain regions examined. In regions of severe myelin deficiency, gliosis, with an increase in astrocytic processes, was prominent. The regional pattern of white matter lesions in bovine ß-mannosidosis was similar to that previously reported in caprine ß-mannosidosis and very different from the pathological changes reported in bovine α-mannosidosis, the most closely related lysosomal storage disease. The specific causal relationship between ß-mannosidase deficiency and myelin deficits has not yet been defined. Supported by NS 20254 to KLL and NS 16886 to MZJ.

482.17

EFFECTS OF CULTURE CONDITIONS AND ENDOCRINE FACTORS ON ANGIOTENSINOGEN PRODUCTION BY RAT PRIMARY ASTROGLIAL CULTURES AND IMMORTALIZED ASTROGLIAL CELLS. K.R.Zahs, L.O.Hong, V.B.Bigornia, S.D.Collins, R.H.Edwards and C.F.Deschepper. Dept of Physiology Box 0444, University of California, San Francisco CA 94143

Rat astroglial primary cultures were found to produce different amounts of angiotensinogen (AOG) according to the brain region from which they were derived. Cultures from pons produced more than cultures from diencephalon, while cultures from cortex produced small or undetectable levels of AOG. A similar regional distribution was observed whether the cells were derived from fetal or neonatal rats. The cultures produced more AOG at 39°C than 37°C, and more in Coons medium than in MEM. AOG production was increased in a dose-related fashion by dexamethasone (DEX) and triiodothyronine (T3). Dibutyryl cyclic AMP (dbcAMP) 10^{-3}M increased AOG secretion and potentiated the effect of DEX, but did not modify the effect of T3. Immortalized astroglial cell lines were obtained by transforming astroglial cells derived from the diencephalons of ld-old rats with the A58 temperature-sensitive mutant of SV-40 large T antigen. At 39°C, selected cell lines displayed astroglial characteristics and secreted AOG in a DEX-sensitive fashion. These cells may therefore be a useful model to study the region-specific expression and endocrine regulation of the AOG gene in rat brains. Supported by USPHS Grants HL29714 and HL38774.

482.18

TRANSGENIC ABLATION OF SCHWANN CELLS DURING DEVELOPMENT. A.Messing, G. Lemke, R.R.Behringer*, J.P.Hammang, R.D.Palmiter*, and R.L.Brinster*. Sch. of Vet.Med., Univ. of Wisc., Madison, WI 53706; Sch. of Vet.Med., Univ. of Penn., Philadelphia, PA 19104; Salk Institute, La Jolla, CA 92037; H.H.M.I., Univ. of Wash., Seattle, WA 98195.

In order to explore cellular interactions during development of peripheral nerve, we generated transgenic mice expressing the diphtheria toxin A chain gene under the control of regulatory elements from the rat P_0 gene. Seventy-eight pups were born from fertilized eggs microinjected with the P_0-DT construct, of which sixteen had integrated the transgene. One of these founder animals, although phenotypically normal, produced transgenic offspring with a generalized hypomyelinating peripheral neuropathy and shortened lifespan. Peripheral nerves of offspring in this line show marked depletion of myelin, but occasional fibers had apparently normal myelin sheaths. Proliferation occurred in the non-myelinating population of Schwann cells. Some non-myelinating Schwann cells had shortened processes and incompletely enclosed bundles of small-diameter axons. These mice should prove useful for studies on the secondary responses of Schwann cells and axons to primary Schwann cell diseases.

482.19

MURINE MONOCLONAL ANTIBODIES SPECIFICALLY KILL A HUMAN GLIOBLASTOMA CELL LINE IN VITRO. C. Matute,[1] V. Sánchez*[1], C. Río*[1], B. Conde*[2] and E. Sinués*[2]; 1-Department of Neurosciences, University of País Vasco, 48940-Leioa; 2-Department of Morphology, University of Zaragoza, 50009-Zaragoza, Spain.

We have produced murine monoclonal antibodies (mAb) to a human glioblastoma cell line (T2) recently established in our laboratory. Balbc mice were immunized using as antigen1-2 millions of T2 cells grown in nude mice . MAbs binding to T2 cells but not normal human central nervous tissue were selected to test their cytotoxic effects on T2 cells in vitro. ^{3}H-thymidine incorporation and total protein content assays showed that mAbs 1A1-B7 and 1D4-G3 were able to alter the normal growth of T2 cells. However, these mAbs did not significantly changed the growth rate of other malignant human cell lines or rat primary astrocytes. An hour incubation with .3 mg/ml of ammonium sulphate precipitated ascitic fluids containing mAb 1A1-B7 followed by .05 mg of rabbit complement applied for two hours was sufficient to kill 74% of T2 cells and 23% of the rat glioma cell line C6, whereas cultured astrocytes grew as in control wells. Irrelevant mAbs, complement itself or a combination of both were not cytotoxic at this concentrations. Preliminary immnunoblot experiments indicate that mAb 1A1-B7 and 1D4-G3 recognize a 200 Kd band. These mAbs bind to a T2 molecule which might show some partial homology to a molecule/s present in rat glioma C6. The mAbs reported here are specific for a human glioblastoma cell line and they might be valuable immunotherapeutical probes.

This work was supported by DGCYT (PM-88-100).

482.20

THE RETINAL PIGMENT EPITHELIUM (RPE) GENERATES AN INCREASE IN SUBRETINAL SPACE VOLUME IN FROG RETINA. B. Huang and C.J. Karwoski. Vision Research Laboratory, Department of Psychology, University of Georgia, Athens, GA 30602.

Membrane impermeable ions, combined with ion-selective electrodes, can be used to measure changes in extracellular space (ΔECS). We have recorded ΔECS in the subretinal space (SRS) in Rana pipiens during normal light-evoked activity. Eyecups were superfused with the ECS marker cations tetramethylammonium (TMA) or tetraethylammonium, or the anions hexafluoroarsenate or α-naphthalene sulphanate.

A light-evoked decrease in concentration of these ions was measured. This decrease is caused by an expansion of the SRS, rather than by uptake mechanisms, because: (1) The amplitude and time course of cation and anion responses are similar; (2) The TMA response recovers to baseline during a long-duration stimulus; (3) The resistance across the SRS is decreased by light.

Glutamate analogues (2-amino-4-phosphonobutyrate+kynurenate or aspartate) do not block the TMA decrease, which indicates mechanisms postsynaptic to photoreceptors are not involved. A TMA decrease is not observed in the isolated retina, therefore the RPE is involved in generating the TMA decrease. The TMA decrease is depressed by Ba^{2+}, a K^+ channel blocker, but is not affected by blockers of anion transport mechanisms.

We conclude that during light the RPE cells shrink, leading to an increase in SRS volume. Light-evoked ΔECS will affect extracellular concentrations of all substances.

Supported by NIH grant EY-03526.

482.21

ISOLATION OF cDNAs EXPRESSED IN THE ADULT MOUSE CHOROID PLEXUS. E. Lecain*, T. Rhyner*, J. Mallet* and B. Pessac, Centre de Biologie Cellulaire, CNRS, 67 rue Maurice Günsbourg, 94205 Ivry sur Seine cedex, France.

We have previously reported the construction of a subtracted cDNA library from a mouse cerebellum astroglial cell clone (Rhyner, T. *et al.*, J. Neurosci. Res., in press). The recombinants isolated from this library, whose sizes range from 100 to 200 bp, have been characterized by nucleotide sequencing and by their pattern of expression in various mouse adult tissues and regions. Two recombinants code for smooth muscle α actin and fibronectin.

The sequences of two other recombinants did not reveal any significant homology with any cDNA described to date when compared with data bases.These recombinants hybridize to mRNAs present in the brain and in various tissues including kidney, testis and muscle, as well as in primary cultures of cerebellar astrocytes. To identify the cell types responsible for mRNA expression, *in situ* hybridization experiments were carried out with RNA probes. The antisens RNAs gave a clear signal in choroid plexuses of the lateral and 4th ventricules as well as in few ependymal cells. In addition, one recombinant hybridized to limited areas of kidney cortex, i.e. some glomerulus cells and the proximal tubule.

These results show that the strategy used in this study has led to the isolation of undescribed cDNAs that hybridize to mRNAs expressed in astrocytes and other cell types outside the CNS.

(Supported by CNRS, MRT, ARC and FRMF).

482.22

EVIDENCE THAT NORMAL ASTROCYTES DO NOT MIGRATE UNDER NON-INVASIVE (NON-TRANSPLANT) CONDITIONS. J.D. Hatton, J.P. Finkelstein & H.S. U*, Div. of Neurosurgery, UC San Diego, La Jolla, CA

Previous research has shown that grafted astrocytes have the ability to migrate throughout most of the rat central nervous system. Reactive host astrocytes have been shown to migrate into superior cervical ganglion autografted into the brain as target tissue. However, the ability of normal astrocytes to migrate under non-invasive conditions has not been explored. To investigate this question, host astrocytes must be labelled without invasive damage. Thus, rats were anaesthetized and placed in a stereotaxic. A burr hole in the skull exposed the dura mater, which was peeled back under a surgical microscope. The pia mater was gently punctured several times with the tip of a 31g needle. The area was overlaid with gelfoam containing fluorescent polyspheres in tissue culture medium. After two hours, the gelfoam was removed and the wound was closed. After 2-4 weks of recovery, rats were perfused with aldehydes and their brains removed and sectioned on a cryostat for fluorescence histology.

Fluorescent polyspheres were taken up by both pial fibroblasts and astrocytes at the pial-glial margin. Labelled astrocytes (identified by GFAP staining) were confined to the area of the original labelling site, and did not migrate either laterally across the pial margin or ventrally into the corticsal layers. Labelled astrocytes did not appear to be either hyperplastic or hypertrophic. Although minimal damage may have occurred during labelling, these results suggest that astrocytes do not migrate in the absence of some invasive stimulus.

GENE STRUCTURE AND FUNCTION IV

483.1

ROLE OF AP2 AND NEGATIVE REGULATORY CIS-ACTING ELEMENT IN GLUTAMINE SYNTHETASE EXPRESSION. H.J. Purohit*, R.G. King and J.F. Mill. Lab. of Molecular Biology, NINDS, NIH, Bethesda, MD 20892.

Glutamine synthetase (GS) plays a central role in nitrogen metabolism and ammonia detoxification in the central nervous system, where its expression is confined to astrocytes. The gene for GS was cloned and the 5′ flanking region isolated. A series of deletion and site-directed mutants of the promoter region were generated by the polymerase chain reaction, cloned into the chloramphenicol acetyl transferase (CAT) expression vector pSV_0CAT, and expressed in various cell lines.

The deletion mutants demonstrate that 421 bp upstream of the transcriptional start site is sufficient for cell type specific expression. This construct is expressed at high levels in primary astrocytes, C6 glioma and HepG2 cultures, and at only low levels in PC12, and HeLa cell lines.

We have identified two regions of importance for the regulation of GS expression, an AP2 site (CCCAGCC) located at -222, and a silencer region (CCTCTGTAG) at -797. Expression of a 251 bp fusion construct with a site-directed mutation in the AP2 site shows an increase when compared to the wild type constructs. When this same mutation is made in a 1086 bp construct containing the silencer region, all expression is suppressed.

483.2

DISTRIBUTION OF KETAMINE-INDUCED FOS IMMUNOREACTIVITY IN RAT BRAIN. R.A. Mueller, K. Gilliam*, L. Grimes, and G.R. Breese. Dept. of Anesthesiol. and Bio. Sci. Res. Cent., School of Med., UNC-CH, Chapel Hill, NC.

To determine the distribution of fos immunoreactivity induced by the dissociative anesthetic ketamine (KET), rats were injected with 40, 80, or 120 mg/kg of KET or 80 mg/kg/ KET plus 10 mg/kg naloxone (NAL) and were perfused 2 hr later with 4% paraformaldehyde plus 0.2% glutaraldehyde. 30 μm Vibratome sections were stained for fos protein. A prominent band of immunoreactive nuclei was detected in posterior but not anterior cingulate cortex. Quantification of intensely stained nuclei revealed maximal expression at 80 mg/kg. NAL attenuated but did not eliminate staining for fos. Other areas exhibiting fos immunoreactivity were: a) central n. amygdala, b) n. Edinger-Westphal, c) paraventricular, parafasicular, medial dorsal, lateral posterior and reuniens n. of thalamus, c) paraventricular, supramammillary, centromedian, and dorsomedial n. of hypothalamus, d) subthalamic n., and e) layers IV and VI of cortex. No staining was noted in neostriatum or hippocampus. These results suggest that KET may alter the metabolism of neurons involved in the affective component of pain perception.

483.3

STRONG EVOLUTIONARY CONSERVATION OF NEUROPEPTIDE Y; CHARACTERIZATION OF SHARK GENE. D. Larhammar, I. Lundell*, and A. G. Blomqvist*. Department of Medical Genetics, Uppsala University, Box 589, S-751 23 Uppsala, Sweden.

Neuropeptide Y (NPY) is an abundant and widely distributed neuropeptide in the CNS and PNS of all mammals investigated. NPY potently influences several physiological parameters, notably blood pressure and food intake which are both increased by NPY. NPY is a member of a peptide family which also includes peptide YY (PYY) and pancreatic polypeptide (PP) as well as the fish pancreatic peptide PY.

To investigate the degree of conservation of NPY at the structural level during evolution, we have recently isolated DNA clones encoding chicken and goldfish NPY. We have previously reported that chick and goldfish NPY reveal a remarkable degree of homology as they display only 1 and 5 differences out of 36, respectively, to human NPY.

Using a chick NPY probe we have now screened a genomic library from horned shark (kindly provided by Dr. G. Litman, Tampa Bay Res. Inst., St. Petersburg, FL). Several clones were found which contain exon two of the shark NPY gene encoding the signal peptide and 34 of the 36 amino acids of mature NPY. Mature shark NPY shows only three differences to the human sequence. The shark sequence displays no unique amino acid residues as compared to the other known NPY sequences. This suggests that shark NPY may be identical to NPY of the common ancestor of cartilaginous fishes, bony fishes, and mammals.

We are now in the process of characterizing lamprey cDNA clones and screening a Torpedo cDNA library.

The impressive similarity of shark NPY to human NPY makes this peptide one of the most highly conserved known; 92% identity.

483.4

POSITIVE AND NEGATIVE REGULATORY ELEMENTS IN THE 5' FLANKING REGION OF THE MUSCLE NICOTINIC ACH RECEPTOR γ-SUBUNIT GENE. B.P. Gilmour and P.D. Gardner. Program in Molecular and Cellular Neuroscience and the Department of Biochemistry, Dartmouth Medical School, Hanover, NH 03756.

AChR expression is profoundly influenced during the development of skeletal muscle. Several lines of evidence suggest that this regulation is mediated in part at the level of transcription. Our laboratory is characterizing transcriptional control elements located in the 5' flanking regions of the AChR subunit genes. Previously we have shown that 720 bp of 5' flanking DNA of the mouse muscle AChR γ-subunit gene are sufficient to direct cell type-specific and developmentally regulated transcription of a reporter gene in transfected muscle cells. To further delineate the elements responsible for this regulated transcription, we have carried out deletion analysis of the 720 bp region. Deletion of a 172-bp region (nucleotides -526 to -355 relative to the transcription start site) resulted in a 2-fold increase in reporter gene expression. Placement of this 172-bp putative repressor region upstream of a heterologous promoter (e.g. SV40) resulted in a 5-fold decrease in reporter gene expression. Further deletion of the 5' flanking DNA (nucleotides -354 to -131 relative to the transcription start site) resulted in a 2-fold decrease in expression. These data suggest the presence of both negative and positive regulatory elements in the 5' flanking DNA.

483.5

Characterization of Type I and Type II $GABA_A$-Benzodiazepine Receptors through Domain Swapping. Sutherland, M., Blake, A., Martin, I.L., Shingai, R., Leech, C.*, Darlison, M.G., Davies, W., Barnard, E.A. Mol.Neurobiol. Unit, Med.Res. Council Cambridge, England, *Department of Zoology, University of Cambridge, Cambridge, England.

Distinct subtypes of the $GABA_A$-benzodiazepine receptor have been characterized in the mammalian CNS using ligands which interact with the benzodiazepine recognition site on this protein. Recent evidence from combinations of $GABA_A$ receptor subunits expressed in transfected mammalian cells suggests that type I benzodiazepine receptors, as characterized by high affinity for CL 218 872 and methyl β-carboline-3-carboxylate are associated with the α_1 subunit, while type II characteristics, which correspond to a low affinity for the above two ligands, are found if α_1 is replaced by α_2 or α_3 subunits in the transfected cells.(1)

In order to delineate these differences further we have exchanged the N-terminal extracellular domain of the bovine $GABA_A$ α_1 subunit with the bovine α_3 subunit and expressed these chimeras in combination with β_1 and γ_2 $GABA_A$ subunits in transfected mammalian cells. The expressed receptors in the cell membranes were characterized by displacement of either [^{3}H] flunitrazepam or [^{3}H] Ro 15-1788 with CL 218 872 or methyl β-carboline-3-carboxylate. Channel integrity and function were studied by electrophysiological analysis. Our results will be discussed in this presentation.References (1) Pritchett, D.B. *et al.* (1989), Science 245, 1389-1392.

483.6

SPECIES-SPECIFIC COMPARISON OF PREPROENKEPHALIN PROMOTERS. R.J. Schroeder, G. Weisinger and E.F. La Gamma, Pediatrics & Neurobiology, SUNY at Stony Brook, NY 11794.

Despite the high degree of homology between the human and the rat preproenkephalin (ppENK) promoters, (89% from +1 to -140, 50 to 60% beyond) expression of this gene varies between tissue and cell type, (La Gamma et al, Int J Devl Neurosci 7:499, 1989). This raises the question, is it the cell background or the DNA sequence that confers this specificity? We made chimeric ppENK-CAT promoter constructs extending to -497, -370, -249, and -152 base pairs from the start site of the rat gene (confirmed by sequencing and restriction analysis). ppENK promoter constructs of the rat and human (pENKAT-12) were transfected into CV1 cells. Transfection efficiency was controlled by Southern analysis of Hirt lysates and beta-galactosidase co-transfection. Basal expression was not detected in any of these constructs. Human ppENK was induced by treatments that increase intracellular cAMP (Forskolin or TPA plus IBMX); similar treatments had no effect on any of the rat constructs. Although the CRE's of the human ppENK promoter are responsible for most of the cAMP inducibility of this gene, this appears not to be the case in the rat. We found five single base pair differences in the region of CRE1 and 2 (Durkin et al, Neurosci Abs, 1990). Sequence differences and/or cell-specific transcription factors, could generate complex patterns of gene expression. Supported by NSF #BNS8719872.

483.7

RAT PREPROENKEPHALIN GENE STRUCTURE: 5′ PROMOTER SEQUENCE DETERMINATION (-1.5 KB) & ANALYSIS. R.C. Durkin, G. Weisinger and E.F. La Gamma, Pediatrics & Neurobiology, SUNY at Stony Brook, NY 11794-8111.

Regulation of gene expression involves the interaction of a multiplicity of factors that bind to specific regions of the gene to influence transcription. Although previous work has focused on the regulation of human preproenkephalin (ppENK) by CRE regulatory sequences close to the transcriptional start site (-70 bp to -133 bp), few studies have investigated DNA regulatory regions further 5′ or for other species. To begin to define other features of gene structure and putative upstream cis-acting DNA elements, we have sequenced up to the BamHI site at -1.5 kb of the rat ppENK gene. Sequence data from -1.5 kb to -370 bp is new information. Additionally we report 5 corrections to the previously published rat sequence in the region of the CRE. Corrections to the sequence in the CRE region could account for species-specific regulatory differences by this element (see R. Schroeder et al, Soc Neurosci, 1990). Additional analysis has revealed 85 to 100% homology with several recognized regulatory motifs including CTF/NF-1, c-Jun/AP1, SP1 etc. Furthermore, several open reading frames potentially coding for polypeptides up to 50 amino acids in length have been noted. Current research seeks to further clarify the biological significance of the 5′ upstream region in basal and induced states by analysis of DNAse I hypersensitivity patterns. NSF grant #BNS8719872.

483.8

CHARACTERIZATION OF THE HUMAN TRYPTOPHAN HYDROXYLASE GENE. K.L. Newell[1,2], K.J. Black*[1,2], L.B. Morgenstern*[1,2], S.L. Winfield*[2], B.M. Martin[2], B.K. Stubblefield*[2], K. Maysak*[2], J.L. Weller*[3], D.C. Klein*[3], and E.I. Ginns[2]. [1]Howard Hughes Medical Institute-NIH, [2]Section on Molecular Neurogenetics, NIMH, and [3]Section on Neuroendocrinology, NICHD, Bethesda, MD 20892.

Tryptophan hydroxylase (EC 1.14.16.4) catalyzes the hydroxylation of tryptophan to form 5-hydroxytryptophan in the pathway of serotonin biosynthesis. As the rate-limiting enzyme in this neurotransmitter pathway, it is important in maintenance of vascular homeostasis, intestinal motility, and central nervous system functions. Tryptophan hydroxylase, along with phenylalanine and tyrosine hydroxylases, comprise a family of aromatic amino acid hydroxylases that require tetrahydrobiopterin and molecular oxygen for the oxidation of the respective amino acids. The structural homology shared by these aromatic amino acid hydroxylases probably reflects their common origin in a single ancestral gene. Although the genes for human and rat phenylalanine and tyrosine hydroxylases have been isolated and characterized, only cDNAs for rabbit and rat tryptophan hydroxylases have been described. We now report the isolation and characterization of genomic DNA for human tryptophan hydroxylase. The genomic DNA is intermediate in size to that encoding tyrosine and phenylalanine hydroxylases, spanning over 15 kb. A Msp I restriction fragment length polymorphism has been identified using a 5' genomic fragment as a probe, and its use for studies of possible involvement of tryptophan hydroxylase in human genetic disorders will be presented.

483.9

SPECIES AND REGIONAL DIFFERENCES IN THE EXPRESSION OF CELL-TYPE SPECIFIC ELEMENTS AT THE HUMAN AND RAT TYROSINE HYDROXYLASE GENE LOCI. G.T. Coker III, K.-Y. Gandelman, M. Moffat*, and K.L. O'Malley. Dept. of Anatomy and Neurobiology, Washington Univ. School of Medicine, St. Louis, MO 63110.

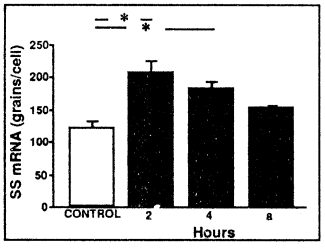
The expression of tyrosine hydroxylase (TH) is confined to different types of neuroendocrine cells. Using a transient assay system, we examined >10 Kb of the human gene and 9.5 Kb of sequence surrounding the rat gene for DNA elements that confer cell-type specific expression. Results derived from the PC12 pheochromocytoma, LAN-1 neuroblastoma, and HepG2 hepatoma cell lines localized cell-type specific sequences for the rat and human genes. Surprisingly, these elements do not appear to be conserved in position or sequence across species. When plasmids containing DNA sequences -749 bp from the transcription start site of the rat gene were introduced into PC12 cells, 2 to 6 fold higher levels of expression were observed compared to the same fragments introduced into HepG2 cells. In contrast to the rat gene, analogous fragments of the human 5' flanking sequence failed to confer cell-type specific expression. However, when plasmids containing a thymidine kinase promoter and either orientation of a 700 bp 3' human gene fragment were introduced into PC12 and LAN-1 cells, we observed a 6 and 3.5-fold increase, respectively, over that observed for HepG2 cells. Deletions of this fragment led to significant activation of transcription, as much as 50-fold over control level. These data suggest the presence of positive and negative elements contributing to the tissue specific expression of tyrosine hydroxylase genes.

mRNA REGULATION: NEUROPEPTIDES

484.1

RAPID STIMULATION BY GROWTH HORMONE OF SOMATOSTATIN mRNA IN THE PERIVENTRICULAR NUCLEUS OF THE ADULT MALE RAT. E Redmond*, P Zeitler*, DK Clifton, and RA Steiner Univ of WA, Seattle, WA 98195

We have recently demonstrated that both SS and GHRH mRNA content in the hypothalamus of adult male rats vary in a manner consistent with the existence of an ultradian rhythm. However, the mechanism underlying this rhythm is unknown. Since previous investigations have demonstrated that GH can stimulate SS mRNA content, at least over a period of days, we tested the hypothesis that GH can stimulate SS mRNA content with a time course sufficiently rapid to account for the ultradian rhythm. Hypophysectomized adult male rats were fitted with jugular catheters, injected with either GH (1 mg) or vehicle and sacrificed at 2, 4 and 8 h after injection (n=4 at each time).



Using *in situ* hybridization, we measured SS mRNA levels in individual neurons of the PeN and compared these levels among experimental groups. We report results (based on ~50 cells per animal) that SS mRNA content is increased at 2 and 4 h after the GH injection and returns to control values by 8 h. **Conclusion:** Rapid feedback of GH on SS gene expression may underlie, at least in part, the ultradian rhythm in SS mRNA content in the hypothalamus of the adult male rat.

484.2

IDENTIFICATION OF PROTEIN-SOMATOSTATIN PROMOTER DNA COMPLEXES IN BRAIN REGIONS SUBJECT TO TIME- AND TISSUE-SPECIFIC SOMATOSTATIN mRNA EXPRESSION C.L. Szymeczek, D.A. Bayliss and D.E. Millhorn, *University of North Carolina, Chapel Hill, NC 27599-7545.*

Somatostatin (SOM) mRNA decreases in rat hypoglossal nuclei (12N) from birth through postnatal day 14 (FASEB J. 3:A391, 1989), after which time it disappears from 12N but remains in other brain nuclei, e.g. nucleus tractus solitarii (NTS). This tissue- and time-specific expression may be due to the presence of different trans-acting regulatory factors (i.e. proteins) in particular brain nuclei at different ages. Therefore, we performed gel shift assays by incubating crude nuclear protein with ^{32}P-labelled fragments of the SOM 5' flanking region. Resultant DNA-protein complexes were examined by polyacrylamide gel electrophoresis. To study time-dependent SOM expression, crude nuclear protein extracts from whole brainstem at ages 0, 7, 14, 21, and 28 days were assayed. To examine tissue-specific SOM expression, crude nuclear protein extracts prepared from adult rat 12N and NTS were used. In both sets of experiments, several specific DNA-protein complexes were identified as far as 750 bases upstream of transcription start site. These findings show that proteins isolated from brain tissue exhibit specific binding to the SOM promoter region. Significance of these DNA-protein interactions in tissue- and time-specific SOM expression in brain is unclear. (NIH HL33813, AHA 881108)

484.3

HALOPERIDOL RAPIDLY INCREASES NEUROTENSIN mRNA LEVELS IN RAT NEOSTRIATUM. K.M. Merchant, M.A. Miller, E.A. Ashleigh* and D.M. Dorsa. GRECC, Seattle VA Medical Center and Depts. of Medicine and Pharmacology, University of Washington, Seattle, WA 98108.

Several recent studies have demonstrated that treatment of rats with antipsychotics such as haloperidol increases neurotensin (NT) immunoreactivity in the neostriatum. The purpose of the present work was to investigate the response of the NT/ neuromedin N gene in striatal cells following acute and chronic treatment with haloperidol. Male Wistar rats were treated with a) a single dose of haloperidol (0.5 mg/kg, i.p.) and were sacrificed 1 h later or b) multiple doses of haloperidol ((0.5 mg/kg/day x 21 d, i.p.) and sacrificed 20-22 h after the last treatment; controls were treated with the vehicle of the drug. In situ hybridization histochemistry using a ^{35}S-labeled antisense riboprobe was employed to determine the level and distribution of NT-mRNA. A computer-assisted image analysis system was used to evaluate the data. In control brains labeled cells were observed in the striatum, nucleus accumbens, median forebrain bundle, olfactory tubercle and the septal nuclei. In anatomically matched striatal sections a sampling of the dorsolateral region showed that there was a dramatic increase in the number (control:42±13, haloperidol:154±30 unilaterally) as well as the optical density of hybridization-positive cells within 1 h after a single dose of haloperidol ($P<0.02$, ANOVA). Such an increase was not observed in the dorsolateral striatum 20 h after the chronic treatment. Thus haloperidol may acutely enhance the expression of the NT/neuromedin N gene in the neostriatum. (Supported by NS 20311).

484.4

HIPPOCAMPAL DYNORPHIN EXPRESSION FOLLOWING SEIZURE, G.L. Yount, J.C. Lauterborn, C.M. Gall and J.D. White, Div. Endocrinology, SUNY, Stony Brook, NY 11794 & Dept. Anatomy and Neurobiology, UC, Irvine CA 92717

The opioid peptides dynorphin (DYN) and enkephalin (ENK) are expressed differentially in dentate granule cells in that DYN is expressed at high levels in the basal state while basal expression of ENK is low. However, following hilus-lesion (HL) induced recurrent limbic seizures, all granule cells also express preproENK mRNA at high levels. In this study we examined preproDYN expression following HL-induced seizures to examine the regulation of expression of co-localized transmitters. Unilateral HL were placed sterotaxically in ketamine-xylazine anesthetized male Sprague-Dawley rats. This procedure reliably produces bilateral electroencephalographic seizures within 90 min of lesion placement that recur for up to 10 hr thereafter. At selected times after HL, animals were killed and dentate gyrus/CA1, CA3, entorhinal and neo-cortical subfields dissected. Total RNA was isolated from these samples and the amount of preproDYN mRNA present in each individual sample was measured by nuclease protection analysis. In dentate gyrus/CA1 samples, preproDYN mRNA levels were found to increase 4-fold and 8-fold above control values by 6 hr and 12 hr post-HL respectively. PreproDYN mRNA levels returned to control values by 24 hr post-HL and were then suppressed 3-fold below control values at 96 hr post-HL. In situ hybridization analyses confirmed that these changes in preproDYN expression occurred in granule cells; additionally, these latter studies also revealed a small population of CA1 pyramidal neurons that were induced to express preproDYN by 48 hr post-HL. Preliminary results suggest that in entorhinal but not neocortex, preproDYN expression was also elevated during recurrent seizure. These findings contrast with those for ENK expression and suggest that different cellular mechanisms regulate expression of these two opioid peptide genes in hippocampal neurons. NIMH 42074, 00801 (JDW); NIH NS 26748 (CMG)

484.5

TRANSSYNAPTIC REGULATION OF ADRENAL PREPROENKEPHALIN: A PARADOX. B.L. Agarwal, J.D. DeCristofaro and E.F. La Gamma, Peds & Neurobiology, SUNY at Stony Brook, NY 11794.

In the adult rat, hypoglycemic-induced transsynaptic mechanisms only partially account for augmented levels of preproenkephalin (ppENK) mRNA (Kanamatsu, PNAS 83:9245, 1986). To validate a transsynaptic process, neonatal rats were made hypoglycemic at a time when functional synapses are deficient. Pups were treated with 20 U/kg of insulin or saline vehicle and sacrificed 24 hours later. ppENK mRNA in medullae from adult rats increased 60-fold but there was no change in the newborn. To prematurely induce functional synaptic activity, thyroid hormone was given to newborn rats on day 2 and 3 of life (Lau, Dev Br Res 44:109, 1988). Controls received saline vehicle or were left unmanipulated. Hypoglycemia resulted in a 20-fold increase in adrenomedullary ppENK mRNA levels only in T3/insulin treated pups. To identify effects of hypoglycemia independent of insulin treatment, intracellular glycopenia was produced by injecting adult rats with 2 deoxy-glucose (500 mg/kg). Similar to the insulin-hypoglycemia group, there was an increase in adrenomedullary ppENK mRNA. We conclude that enkephalin biosynthesis like co-stored catecholamines appears to be induced by a transsynaptic process. However, decreasing transsynaptic activity by denervation or ganglionic blockade also increases ppENK mRNA, a paradox which remains unexplained. Supported by the March of Dimes Foundation.

484.6

NATURE AND DISTRIBUTION OF PREPROTACHYKININ mRNAS IN HUMAN BASAL GANGLIA. M.S. Poosch, D.M. Haverstick, A. Mandal*, I.C. Xue, K. Shibata, L.J. Dragovic* and M.J. Bannon. Center for Cell Biology, Sinai Hospital, and Wayne County Medical Examiner's Office, Detroit, MI 48235.

Preprotachykinin (PPT, i.e. substance P/neurokinin A-encoding) mRNAs in human basal ganglia tissues obtained at autopsy were quantitated using various human PPT cDNA clones isolated from basal ganglia (courtesy of T. Bonner, NIMH) or hypothalamic cDNA libraries. The size of PPT mRNAs by Northern blot analysis was approximately 1300 bases, confirming the postmortem stability of these mRNAs. Gross anatomical analysis indicated a roughly even distribution of PPT gene expression throughout the entire human caudate-putamen but an absence of PPT mRNAs in the globus pallidus. Solution hybridization (RNA protection) experiments established that approximately 80% of PPT mRNA was beta-PPT (full length) and 20% gamma-PPT (minus exon 4) mRNA, with no detectable alpha-PPT (minus exon 6) mRNA. Thus the proportion of various PPT mRNAs encoding different combinations of tachykinin peptides in human basal ganglia differs significantly from bovine and rodent tissues. The quantitation of PPT primary transcript versus mature PPT mRNA is currently being undertaken using an incompletely spliced hypothalamic PPT cDNA clone containing both exon and intervening sequences.

484.7

DIFFERENTIAL AFFECTS OF ADRENALECTOMY ON NPY mRNA LEVELS IN THE ARCUATE NUCLEUS AND BRAIN STEM. B.D. White, R.G. Dean* and R.J. Martin. Dept. of Foods and Nutrition, University of Georgia, Athens, GA 30602

Neuropeptide Y (NPY) is a potent inducer of food intake. Its site of action is thought to be at the paraventricular nucleus (PVN) of the hypothalamus. Cell bodies of NPY nerve terminals in the PVN are located within the arcuate nucleus of the hypothalamus and within various nuclei of the brain stem. We have previously shown that adrenalectomy (ADX) decreases NPY mRNA levels in some regional brain areas (in press, Neurosci. Lett.). To determine if ADX decreases NPY mRNA levels in areas which innervate the PVN, we examined the affect of ADX on NPY mRNA levels in the arcuate nucleus and brain stem. Thirty rats were divided into three groups: ADX, ADX + corticosterone, or Sham. Four days after surgery, tissues were taken and the total RNA isolated. Dot blots were made and hybridized with a cRNA probe to rat NPY mRNA. NPY mRNA was quantitated by densitometry. ADX decreased NPY mRNA levels in the arcuate nucleus by 50% ($p<0.05$). The decrease was reversed by corticosterone replacement. ADX had no affect on NPY mRNA levels in the brain stem. These results suggest that glucocorticoids may have a differential affect on the gene expression of NPY in the arcuate nucleus and brain stem. This implies that a regulatory difference may exist between the arcuate nucleus and brain stem in the control of NPY levels in the PVN.

484.8

ALTERED NEUROPEPTIDE GENE EXPRESSION IN EXPERIMENTAL DIABETES. P.Fernyhough, W.J.Smith* and D.R.Tomlinson*. Dept. of Pharmacology, St. Bartholomew's. Medical College, London EC1M 6BQ, U.K.

The aim was to determine whether altered levels of substance P in diabetic rats could be explained by changes in gene expression of the precursor protein, preprotachykinin (PPT). Rats were studied 4 wk after induction of streptozotocin (50 mg/kg i.p.) diabetes. Dorsal root (L_4 and L_5) and trigeminal ganglia were removed rapidly at death and total RNA isolated. Northern blots were prepared and probed using a [^{32}P]-labelled 39-mer oligonucleotide probe complimentary to PPT. Portions of sciatic nerve (1 cm) were removed from the same rats at death, snap frozen in liquid N_2, substance P extracted (< 2h later), and substance P-like immunoreactivity (SPLI) was measured by radioimmunoassay. Sciatic nerve from diabetic rats contained much less SPLI (46.8±5.6 [sem] pg/cm) than segments from control rats (87.4±12.5 [sem] pg/cm; $p < 0.01$). This was associated with markedly reduced levels of the PPT mRNA (relative to total RNA) in the dorsal root ganglia of the diabetic rats compared to control rats. PPT mRNA levels in trigeminal ganglia were, however, greatly increased in diabetic rats compared with control rats. These findings indicate that fundamental changes in gene expression for substance P occur in experimental diabetes.

484.9

CHARACTERIZATION OF A NUCLEAR PROTEIN BINDING SITE IN THE 5' FLANKING REGION OF THE MOUSE POMC GENE. J.F. Bishop and M.M. Mouradian, Experimental Therapeutics Branch, NINDS/NIH, Bethesda, MD 20892.

Several protein binding sites have been identified in the 5' flanking region of the mouse POMC gene using AtT-20 cell nuclear extracts in exonuclease protection assays. In this study, the binding site located between -137 and -131 was further characterized because of its sequence homology (TCAGCCA) to AP-1 binding site (5 of 7 bases), and inducibility of POMC mRNA in these cells by phorbol esters. A double-stranded oligonucleotide probe containing 3 copies of this sequence was used in gel retardation experiments with AtT-20 cell nuclear extract. A heat labile factor(s) exhibited specific binding which was abolished by a 50-fold excess of unlabeled homologous DNA, and was unaffected by oligos containing other known transcription factor binding sites, including AP-1, CTF/NF-I and Pit-1. These observations suggest that a major exonuclease protection site with homology to AP-1 site in the promoter region of the mouse POMC gene is a target for an as yet unidentified nuclear protein which has DNA binding characteristics different than those of authentic AP-1.

484.10

OXYTOCIN mRNA IS PRESENT IN THE RAT NEUROINTERMEDIATE LOBE

Brooks, P.J., Lund, P.K., Caldwell, J.D., Jirikowski, G.F., and Pedersen, C.A. The Neurobiology Curriculum and the Departments of Physiology and Psychiatry , University of North Carolina at Chapel Hill, Chapel Hill, NC

The mRNAs encoding vasopressin (AVP) and oxytocin (OXY) are synthesized in cells located in the paraventricular and supraoptic nuclei. At last years Neuroscience meeting, two groups (Lightman et al, 142.5, McCabe et al, 142.8) presented evidence indicating the presence of AVP mRNA in the posterior pituitary. In the present experiment, we used a solution hybridization–ribonuclease protection assay to detect OXY mRNA in the rat neurointermediate lobe.

Pituitary glands were removed from lactating female rats and dissected into anterior (AL) and neurointermediate (NIL) lobes. Total RNA was isolated from the tissues and hybridized in solution with a 162 base 32P labelled cRNA probe transcribed from plasmid PGEM4–OXY3c (plasmid provided by Dr. Tom Sherman, Univ. of Pittsburgh). After hybridization and ribonuclease treatment, the protected products were analyzed by polyacrylamide gel electrophoresis.

A protected band corresponding to OXY mRNA was seen in RNA samples from the NILs, but not from the AL. This result indicates that, like AVP mRNA, OXY mRNA is also present in the rat NIL. Ongoing experiments are designed to determine whether NIL OXY mRNA may be present in axons from magnocellular OXY neurons.

485.1

CALCIUM IN DROSOPHILA NEURONS IN CULTURE. W. Dale Branton and Marla S. Rudnick*. Department of Physiology, University of Minnesota, Minneapolis, MN 55455.

Using the fluorescent [Ca^{++}] indicator fura 2, we have imaged [Ca^{++}] in neurons which were allowed to differentiate in culture from disassociated 4-5 hr *Drosophila* embryos. We could consistently produce a rise in [Ca^{++}] levels in cell bodies, processes and growth cones by either raising external [K^+] or by application of toxins from scorpion venom (*Tityus serrulatus*). The scorpion toxins produce high rates of firing of action potentials in neurons via effects on voltage dependent sodium channels. In either case the rise in [Ca^{++}] could be prevented by zero [Ca^{++}], 1 mM EGTA in the solution bathing the cells. The scorpion toxin-induced rise in [Ca^{++}] could also be prevented by TTX, which prevents the depolarizing action of the toxin by blocking sodium channels. Preliminary results indicate that pre-incubation of the cells with *Plectreurys* toxin (PLTX, Branton et al, J. Neurosci 7(12):4195-4200, 1987) can prevent virtually all the rise in [Ca^{++}] in the soma, processes and growth cones. PLTX has been previously shown to block Ca^{++} currents in these cells measured under voltage clamp in whole cell patch configuration (Leung et al, Neuron 3:767-772, 1989). These findings with fura are consistent with the previous findings and support the idea that the currents measured in whole cell patch are representative of currents in intact neurons. We are also investigating *Hololena* toxin (HoTX, Bowers et al PNAS 84:3506-3510, 1987), a selective blocker of non-inactivating Ca^{++} currents in these cells (Leung et al, Neuron 3:767-772, 1989), to determine if its action is spatially selective.

485.2

HIGH POTENCY NIMODIPINE EFFECTS ON WHOLE CELL CALCIUM METABOLISM: SINGLE CELL, POPULATION RESPONSES AND OSCILLATORY BEHAVIOR IN GH CELLS. J. Chisholm, J. Davis* and V. Diaz*. Inst. Preclin. Pharmacol., Miles Inc. West Haven, CT 06516

Single-cell ionized cytosolic calcium (Ca_i) responses to depolarization (high K^+), measured with fura-2, were potently inhibited by nimodipine (NIM) in the highly differentiated clonal GH_3 cell line. Individual cells which responded to high K^+ were qualitatively similar to cell population responses: basal Ca_i (150nM) increased to an initial high peak phase (over 400nM), followed by an extracellular Ca^{2+} (Ca_e)-dependent plateau phase (200 nM), persisting more than 30 min in the absence of NIM. A majority of cells (60%) also fired NIM sensitive spontaneous oscillatory Ca_i spikes, heterogenous with respect to both amplitude (up to 200 nM) and frequency (up to 0.2 Hz). Some cells with stable Ca_i developed spiking activity and/or reverted spontaneously. Oscillations were inhibited under depolarized conditions without drug. The high K^+-induced sustained influx of Ca_e was completely eliminated by NIM, both in cell suspensions (<10 nM), and in single cell determinations in monolayer (addition <50 nM). This correlated with potent NIM block of high K^+ induced cell-associated ^{45}Ca (IC_{50}=0.2 nM). These data support both the physiological significance of sustained Ca^{2+} influx to whole cell calcium metabolism, and the functional activity of NIM at nanomolar concentrations on plasma membrane Ca conductance.

485.3

DEPOLARIZATION-INDUCED CHANGES IN CYTOSOLIC FREE CALCIUM CONCENTRATION IN ISOLATED NERVE TERMINALS FROM RAT POSTERIOR PITUITARY. J. T. Russell, Section on Neuronal Secretory Systems, LDN, NICHD, Bethesda, MD 20892.

Nerve endings (neurosecretosomes), isolated from rat posterior pituitaries were placed on protamine coated coverslips and perfused with balanced salt solutions. Cytosolic free calcium concentration in these nerve terminals was measured by monitoring the fluorescence of the calcium indicator, fura-2 using high resolution video microscopy. Intracellular calcium ion concentration rapidly rises in some of the terminals upon perfusion with solutions containing elevated potassium concentration (25 to 55 mM K_o) or veratridine (60 μM). The initial rise is followed by a slow decline in $[Ca^{2+}]_i$ to resting levels. The depolarization-induced increase in $[Ca^{2+}]_i$ was inhibited by the presence of nimodipine, and ω-conotoxin, two voltage-dependent calcium channel antagonists. Removal of sodium from the perfusion medium also caused a rapid rise in the intracellular calcium levels presumably due to the abolition of sodium-calcium exchange.

485.4

ACTIVITY-DEPENDENT INCREASES IN $[Ca^{2+}]_i$ IN CULTURED RAT SEPTAL NEURONS.

D. Bleakman, [1]N.L. Harrison, J.D. Zucker, B.H. Wainer & R.J. Miller. Dept. of Pharmacological & Physiological Sciences and [1]Dept. of Anesthesia and Critical Care, Univ. of Chicago, Chicago, IL. 60637.

We have begun to study the cellular processes which are involved in the buffering of physiological Ca^{2+} signals in CNS neurons. Simultaneous whole-cell patch clamp recordings and fura-2 based microfluorimetric recordings of $[Ca^{2+}]_i$ were made from cultured rat septal neurons at 25°C. Neuronal resting potentials were -55 to -65mV, input resistances 200-800MΩ and basal $[Ca^{2+}]_i$ values 130 ± 20nM (n=13). Neurons were held at -60mV by constant current injection and action potentials (AP's) evoked by brief depolarizing current pulses.

Trains of AP's elicited increases in $[Ca^{2+}]_i$ that returned to basal values with approximately exponential kinetics ($\tau \approx 3$s.). High frequency (≥ 25Hz) trains of AP's produced larger increases in $[Ca^{2+}]_i$ than low frequency (≤ 10Hz) trains for a given number of AP's, indicating a significant decline in the $[Ca^{2+}]_i$ between successive AP's at lower frequencies. The relationship between the number of AP's and $[Ca^{2+}]_i$ approached an asymptote at high $[Ca^{2+}]_i$ loads as previously observed in rat dorsal root ganglion cells (DRG) (S.A. Thayer and R.J. Miller, J. Physiol., in press). However, this asymptote was reached at 100-150 AP's compared to 20-60 AP's in DRG cells. Preliminary results suggest that both mitochondrial buffering and Na/Ca exchange mechanisms contribute to the recovery of the rise in $[Ca^{2+}]_i$ following activity in cultured rat septal neurons.

485.5

SPATIAL AND TEMPORAL RESOLUTION OF CALCIUM TRANSIENTS PRODUCED BY STIMULATION OF DIFFERENTIATING AMPHIBIAN SPINAL NEURONS. J.Holliday[1], R.J.Adams[2], T.J.Sejnowski[2], and N.C.Spitzer[1]. Dept. of Biology and Center for Molecular Genetics[1], University of California, San Diego, and Laboratory for Computational Neurobiology[2], Salk Institute, La Jolla, CA.

Action potentials evoked from *Xenopus* spinal neurons exhibit a calcium dependence at initial stages of differentiation that shifts to a sodium dependence over a 24 hr period (Spitzer and Lamborghini, 1976). Therefore, stimulation is expected to produce greater calcium influx at early stages of differentiation than during later periods of development. However, the intracellular free calcium level attained upon stimulation is likely to be influenced by other factors such as the release of calcium from intracellular stores and calcium sequestration and buffering. These variables may also change over development.

Changes in the intracellular calcium levels produced by depolarization have been examined in intact (non-dialyzed) cultured neurons using the calcium indicator, fluo-3 and high speed confocal image analysis. Elevations of intracellular free calcium in response to depolarization occur throughout the cell, most notably in the nucleus. The initial onset of increased indicator fluorescence is not detectably delayed in the nucleus relative to the cytosol under measurement conditions with a time resolution as fine as 2 msec. The fluorescence increases to peak values within approx. 200 msec. The initial fluorescence decay was fitted to first order kinetics with a rate constant of about -0.15 sec^{-1}. After stimulation-evoked increases began to decline, slower elevations in fluorescence occurred. The contributions of calcium release from intracellular stores and calcium buffering to observed changes in intracellular calcium are considered. The effects of neuronal maturation on patterns of stimulus-evoked fluorescence will be described.

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485.6

CA^{++} STORES IN PURKINJE CELLS: LOCALIZATION OF $INSP_3$ RECEPTOR AND CALSEQUESTRIN. K. Takei*, A. Metcalf*, G. Mignery[1*], P.Volpe[2*], T. Südhof[1*], and P. De Camilli. Dept. Cell Biol. Yale Univ. Med. Sch., New Haven, CT; [1]Dept. Mol. Gen. and HHMI, Univ. Texas, SWMC, Dallas, TX; [2]Dept. Phys. Biophys. Univ. Texas Med. Branch, Galveston, TX.

It is now well established that intracellular Ca^{++} stores play an important role in cellular regulation. Ca^{++} is stored within membranous organelles from which it can be released by second messengers. A large fraction of Ca^{++} within these stores is bound to Ca^{++}-binding proteins which in the striated muscle and chick cerebellum include calsequestrin. To further elucidate the nature of Ca^{++}-storing organelles in neurons, we have carried out a comparative analysis of the distribution of the InsP3-gated Ca^{++} channel (IP3R) and calsequestrin immunoreactivity in cerebellar Purkinje cells (PC).

As previously observed by us (Nature 342: 192-195, 1989), immunogold labeling of PC revealed a widespread distribution of the IP3R throughout the smooth endoplasmic reticulum (sER), including tubules and cisternae present in dendritic spines and axon terminals. In addition, peculiar stacks of sER cisternae were frequently observed in all regions of the PC cytoplasm. Portions of these cisternae apposed to each other were particularly enriched in IP3R (for the concentration of the IP3R at these sites see also Satoh et al. JCB in press), and appeared to be connected by feet-like structures reminiscent of those connecting sarcoplasmic reticulum and T-tubules at muscle triads.

Calsequestrin immunoreactivity was concentrated selectively in a subset of PC smooth surfaced tubules and vesicles which appeared to be part of the sER but was not enriched in the lumen of InsP3 receptor-rich stacks of sER cisternae. In addition, it was not detectable in the rough ER. These findings are consistent with the existence of structurally specialized subcompartments in Ca^{++} storing organelles.

485.7

INOSITOL 1,4,5-TRISPHOSPHATE ACTIVATES A PUTATIVE CALCIUM POOL-REFILL CHANNEL IN *XENOPUS* OOCYTES. B. Gillo, S. Mundamattom, S.C. Sealfon Dept. of Neurology and Fishberg Center in Neurobiology. Mount Sinai Medical School. New York, N.Y. 10029

Plasma membrane inositol phospholipid-operated calcium channels have previously been found in lymphocytes and mast cells and postulated to be involved in filling endoplasmic reticulum calcium stores. We demonstrate in intact *Xenopus* oocytes that inositol 1,4,5-trisphosphate (IP_3) activates a calcium influx (measured via the native calcium activated chloride current) which is antagonized by cadmium with an IC_{50} of about 200μM. This conductance was insensitive to the VSCC antagonists verapamil and amiloride. Measurement of the conductance changes and reversal potential in the presence of barium ions supports the existence of a native IP_3-activated calcium channel ($Ca_{(IP3)}$) in the oocyte. We find that the opening of this channel is accompanied by refilling of the IP_3-sensitive calcium pool from the extracellular medium. We propose that a second messenger-operated calcium channel may contribute to the refill of IP_3-releasable intracellular calcium stores.

485.8

GENERATIONAL ACETALDEHYDE (AlCHO)EFFECT ON MITOCHONDRIAL CNS Ca^{2+} UPTAKE. E. Egaña & M.T. Ramirez* University of Chile; Faculty of Medicine; Institute of Experimental Medicine; Laboratory of Neurochemistry; Santiago 7-Chile

It has showed in several models of experimental alcoholism that AlCHO the first product of EtOH oxidation reaches all organs, including several CNS areas all connected to metabolism of the toxic. The cell membrane is one of the target structure of deletereous effect; in neuronal tissue affects transport, depolarization, neurotransmittion, neurosecretion, etc. most of these processes linked to Ca^{2+} transmembrane transport. Our Institute has created an experimental model whose rats during seven generations were expossed to AlCHO along their lives from embrion to adult including crossing (Egaña et al. since 1987).

Adult albino Wistar rats♂ & ♀Normal and "AlCHO/G rats these last were injected dayly 200 mg/Kg rat AlCHO i.p. during their life; intrauterine, lactation (by mother injection), inmature and adult age, during 7 generations. Five mitochondrial CNS' areas were studied: brain cortex, hypothalamus, hyppocampus, cerebellum & midbrain. Site I mitochondrial electron transport and Ca^{2+} influx were studied with a polarigraph and "ad hoc" selective electrod in a 4.5 ml chamber with adequate reactional medium.

Results showed that Ca^{2+} uptake was decreased in "AlCHO/G rats" compared with normal, particularly when hypothalamus, hyppocampus and cerebellum were studied.

485.9

PHOTOLYSIS OF THE CAGED Ca^{2+} CHELATOR DIAZO-4 RAPIDLY DECREASES Ca^{2+} ACCUMULATION AND Ca^{2+} CHANNEL INACTIVATION IN *APLYSIA* NEURONES. M.W. Fryer and R.S. Zucker. Dept. of Mol. & Cell Biology, Univ. of Calif., Berkeley, CA 94720.

Ca^{2+}-mediated Ca^{2+} channel inactivation in *Aplysia* neurones is seen as either a decline of Ca^{2+} current during long depolarizing pulses or as the loss of peak current in a test pulse (P2) following a pre-pulse (P1). We used the photolabile Ca^{2+} chelator diazo-4 (Adams et al., J. Am. Chem. Soc., 211, 7957, 1989) to generate rapid decreases in intracellular free Ca^{2+} ($[Ca^{2+}]_i$) under both sets of conditions. Left upper quadrant neurones from the abdominal ganglion were injected with a diazo-4/CsCl mixture to a final diazo-4 concentration of 2-5mM. When a brief, intense light flash was given during a long depolarizing step the decline of Ca^{2+} current was markedly slower than the control. Separation of the overlayed current relaxations was apparent after approximately 10ms. In paired-pulse experiments, a light flash given in the inter-pulse interval increased the P2/P1 peak Ca^{2+} current ratio and hence accelerated the recovery from inactivation. The results show that diazo-4 may be used to rapidly buffer the high local $[Ca^{2+}]_i$ that is present near Ca^{2+} channels during and after activity. The kinetics of such changes are somewhat more rapid than expected from Ca^{2+}-dependent enzymatic mechanisms of inactivation development and recovery. Supported by NIH Grant NS 15114 and NH&MRC (Aust.) C.J. Martin fellowship to MF.

485.10

CONTROL OF NEURONAL CALCIUM CURRENT BY INTRACELLULAR CALCIUM. B.D. Johnson* and W.L. Byerly. Dept. of Biol. Sci., University of Southern California, Los Angeles, CA 90089.

It has been suggested that molluscan neuronal calcium channels are modulated intracellularly both by cAMP- and Ca^{2+}-dependent processes. Using an internal perfusion, voltage clamp technique on isolated Lymnaea neurons, we have identified a Na^+ current activated at the resting potential by a combination of cAMP and theophylline, which appears in the absence of any effect on the voltage dependent calcium (I_{Ca}) or potassium (I_K) currents. The magnitude of this current can be reversibly reduced if ATP is not added to the internal perfusate, suggesting that cAMP-dependent phosphorylation may selectively modulate this current over I_{Ca} and I_K. In contrast, I_{Ca} is modulated by internal calcium. Inactivation, as measured by a double-pulse paradigm, is reversibly enhanced by decreasing the concentration of intracellular calcium buffer (EGTA), and the long-term disappearance of I_{Ca} (washout) is increased with a larger calcium load. Maximal calcium currents, stimulated every six seconds with 500ms pulses, increased the rate of washout over four times, as compared to cells monitored with 20ms pulses every minute. We hope to further delineate calcium's role in regulating I_{Ca} using photorelease of caged Ca^{2+} to produce sudden increases in intracellular Ca^{2+}.

485.11

EXOGENOUSLY-ADDED ATP PROMOTES ^{45}Ca INFLUX IN PC-3M CELLS. M.A. Oleshansky, M.L. Koenig, M.A. Jackson*, and J.B. Trepel*[1] Dept. of Medical Neurosciences, Walter Reed Army Institute of Research, Washington DC 20307 and [1]Lab of Pathology, NCI, Bethesda, MD 20892.

It has been reported recently that addition of ATP to the extracellular medium can transiently increase cytosolic Ca^{2+} concentrations in cultured neuronal cells (J. Neurochem., 50:295-301) and is associated with synaptic potentiation in hippocampal slices (Brain Res., 491:356-9). To further characterize the effect of exogenous ATP on Ca^{2+} homeostasis, we have studied PC-3M cells, a prostate tumor cell line which demonstrates a large transient increase in free intracellular Ca^{2+} in response to added ATP. We now show that at least part of the ATP-dependent increase in intracellular Ca^{2+} levels in PC-3M cells can be attributed to an enhanced influx of Ca^{2+}. The ATP-stimulated uptake of ^{45}Ca is saturable, does not appear to be voltage-gated, and is relatively slow ($t_{1/2}$=5 min). The response is dose-dependent with maximal influx at an added ATP concentration of 3mM. Influx is markedly reduced at higher concentrations of the purine. Preliminary experiments with verapamil and a number of inorganic Ca channel antagonists suggest that the influx occurs via an ion channel. Dihydropyridine channel blockers appear to be unable to block the ATP-stimulated uptake of Ca^{2+}. These results indicate that ATP is able to alter Ca^{2+} homeostasis by a direct effect on a non-voltage gated Ca^{2+} channel.

486.1

CALCIUM CHANNEL REOPENINGS AT RESTING MEMBRANE POTENTIALS FOLLOWING PRIOR DEPOLARIZATION.

P. A. Slesinger & J. B. Lansman. Graduate Neuroscience Program, Univ. of Calif. Med. School. San Francisco, CA 94143.

Neurotransmitter release depends critically on Ca influx through voltage-dependent Ca channels. Although Ca current is inhibited by a wide variety of substances, mechanisms for potentiation of Ca current are less well known. Unitary Ca currents were recorded with 90 mM Ba in the pipette from mouse cerebellar granule cells grown *in vitro*. Granule cells possess a single class of dihydropyridine-sensitive Ca channels (21 pS) that open with depolarizations positive to -30 mV (V_h=-100 mV). Depolarizations to ~0 mV for 100 ms elicit brief openings with a mean open lifetime of ~0.5 ms. Following the termination of the voltage step, Ca channels close with a rate too fast to be detected at -70 mV. By contrast, two types of Ca channel activities are observed following termination of very positive prepulses (>+50 mV): channels that remain open at the end of the prepulse and channels that close, but reopen briefly after a delay. We analyzed the kinetics of Ca channels that were open following the prepulse. Distribution of open time durations for all openings following the prepulse was fit by a single exponential with a τ=4.5 ms. Distribution of latencies to first reopening was fit by a single exponential with a τ=7.7 ms. With shorter, positive prepulses (<25 ms), fewer reopenings are produced and the time constant for the first latency histogram was faster. Thus, Ca channel reopenings depend on both the amplitude and duration of prepulse. These results are consistent with Ca channels returning from an inactivated or blocked state and suggest that following a train of action potentials, the opening of Ca channels at membrane potentials far from the equilibrium potential for Ca would produce a large component of Ca influx.

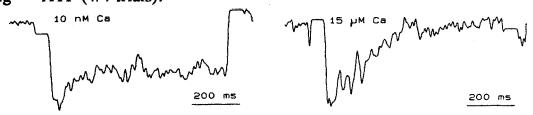
486.2

CALCIUM AND CALCINEURIN INCREASE THE INACTIVATION RATE OF L-TYPE CALCIUM CHANNELS IN PLANAR BILAYERS.

Y. Hirschberg, P.A. Koplas & R.L. Rosenberg. Curr. in Neurobiology and Dept. of Pharmacology, Univ. of N. Carolina, Chapel Hill, NC 27599.

Inactivation of L-type Ca channels is voltage- and Ca-dependent. To study Ca-dependent inactivation, we reconstituted cardiac and neuronal L-type Ca channels into planar bilayers where it was possible to control internal Ca^{2+} and Ca-dependent proteins. Inward Ba currents were evoked by 800 ms pulses to 0 mV, and inactivation timecourses were observed in ensemble averages of single-channel records. The stimulatory G-protein, G_s, and DHP agonist 202-791 were included to increase channel lifetime and open time.

In contrast to previous reports (RLR et al.,1988, JGP 92;27), reconstituted L-type Ca channels did express Ca-dependent inactivation. Addition of 15-500 μM Ca^{2+} to the internal solution increased the inactivation rate in 5/11 experiments (see Fig.). Addition of calcineurin (0.1 mg/ml) and Ca^{2+} (5-500 μM) increased the inactivation rate in 5/7 experiments where increased Ca^{2+} alone had no effect. There was no effect of calcineurin in the absence of Ca^{2+}. This suggests that calcineurin and similar proteins in the membrane preparations can be activated by Ca^{2+} and increase the rate of Ca channel inactivation. The Ca-dependent inactivation of reconstituted channels (with or without calcineurin) did not appear to result from dephosphorylation, because the effect of Ca^{2+} was reversible even in the absence of Mg^{2+}-ATP (4/4 trials).



486.3

MONOCLONAL ANTIBODIES TO THE ω-CONOTOXIN-SENSITIVE CALCIUM CHANNEL FROM RABBIT BRAIN MEMBRANES

Junshi Sakamoto, K.K. Stang* and K.P. Campbell. Howard Hughes Medical Institute and Dept. of Physiology and Biophysics, University of Iowa College of Medicine, Iowa City, IA 52242.

The high affinity ω-conotoxin receptor of the calcium channel from rabbit brain membranes has been characterized using monoclonal antibodies. Mice were initially injected with isolated rabbit brain membranes and boosted with heparin-agarose eluate from rabbit brain membranes, which was enriched in the ω-conotoxin receptor. Hybridoma supernatants were screened for immunoprecipitation of ω-conotoxin binding activity. Monoclonal antibodies from hybridoma culture supernatants were preincubated with goat anti-mouse IgG-Sepharose beads (GAM beads) to form monoclonal antibody GAM beads. These beads were then tested for their ability to immunoprecipitate the ^{125}I-ω-conotoxin-prelabeled receptor from CHAPS-solubilized rabbit brain membranes. Three monoclonal antibodies were found to specifically immunoprecipitate the ω-conotoxin-labeled receptor. The ω-conotoxin sensitive calcium channel has been isolated from total rabbit brain membranes using a combination of heparin-agarose chromatography, sucrose gradient centrifugation, and WGA-Sepharose chromatography. The monoclonal antibodies specifically precipitating ω-conotoxin binding activity are being used to characterize and identify the subunit composition of the ω-conotoxin-sensitive calcium channel isolated from rabbit brain membranes. K.P. Campbell is an Investigator of the Howard Hughes Medical Institute.

486.4

Isolation of a Rabbit Brain Transcript with Homology to the β Subunit of the Skeletal Muscle Dihydropyridine Sensitive Calcium Channel.

M. Pragnell, S.D. Jay, C.J. Leveille and K.P. Campbell Howard Hughes Medical Institute, Neuroscience Program and Dept. of Physiology and Biophysics, University of Iowa College of Medicine, Iowa City, IA 52242.

The dihydropyridine sensitive calcium channel, enriched in rabbit skeletal muscle T-tubules, consists of four subunits (α_1, α_2, β and γ). All of these subunits have been recently cloned. Sequence information suggests that α_1 is the principle transmembrane subunit which contains the pore of the ion channel. The cDNA sequence of the β subunit predicts numerous consensus phosphorylation sites (Ruth et al., Science 245:1115) consistent with in vitro studies suggesting a regulatory role. We have isolated a cDNA for the γ subunit (Jay et al., Science 248:490) and the β subunit. cDNA probes derived from the skeletal muscle β subunit message have been shown to crosshybridize with a 3 Kb mRNA species in rabbit brain. This is significantly larger than the 1.8 Kb message observed in skeletal muscle. We have screened a rabbit brain cDNA library with probes made from the skeletal muscle β subunit cDNA clone. A 0.5 Kb and a 2.2 Kb insert were isolated that crosshybridized with the skeletal muscle β sequence. These inserts were then used as probes to rescreen the library and isolate full length cDNA clones. Following completion of sequence analysis a comparison will be made between the predicted structure of the encoded protein and that of the skeletal muscle β subunit. This will test the hypothesis that a homologous brain protein exists which has a calcium channel regulatory function analogous to that proposed for the skeletal muscle β subunit. K.P. Campbell is an investigator of the Howard Hughes Medical Institute.

486.5

PRIMARY STRUCTURE AND EXPRESSION OF THE RAT BRAIN CLASS-A CALCIUM CHANNEL.

T.V. Starr, W.A. Prystay* and Terry P. Snutch. Biotechnology Laboratory, Univ. of British Columbia, Vancouver, B.C., Canada V6T 1W5.

We have isolated a number of rat brain cDNAs which are homologous to the alpha-1 subunit of the dihydropyridine receptor/Ca channel of rabbit skeletal muscle and heart. Preliminary analysis shows that at least four distinct classes of Ca channel genes are expressed in rat brain. Currently, we are investigating the functional and molecular relationships between the different neuronal Ca channel family members.

Using Northern blot analysis, rat brain class A cDNAs (rbA) hybridize to two major transcripts of approx. 8.3 and 8.8 kilobases (kb). Partial DNA sequence analysis of 20 class A cDNAs indicates that these clones fall into two subfamilies that differ at their 3' ends. One class A clone, rbA-126, has been entirely sequenced and contains a single open reading frame of 6.9 kb. The deduced amino acid structure of rbA-126 shows that it contains four repeated homology domains, each containing six putative transmembrane segments. The four repeated domains share approx. 55% amino acid identity with the cardiac Ca channel. In addition, rbA-126 possess two large hydrophyllic domains not related to sequences found in previously cloned Ca channels. One of these is a 470 amino acid segment that separates domains II and III. The second is an approx. 600 amino acid region which follows domain IV. The significant structural differences between rbA-126 and other cloned Ca channels suggest the possibility that functional differences may also exist.

486.6

PRIMARY STRUCTURE AND EXPRESSION OF THE RAT BRAIN CLASS-B CALCIUM CHANNEL.

S.J. Dubel and T.P. Snutch. Biotechnology Laboratory, Univ. of British Columbia, Vancouver, B.C., Canada V6T 1W5.

We have found that rat brain expresses a family of genes (Class A, B, C and D) that are related to the alpha-1 subunit of skeletal muscle and cardiac Ca channels (Snutch et al, PNAS 1990). Northern blot analysis reveals that a set of cDNAs designated rat brain class B (rbB) hybridize to a brain transcript of approx. 10 kilobases (kb). A series of overlapping cDNAs were isolated, sequenced and found to contain a single open reading frame of 6.8 kb. The predicted rbB peptide is most closely related to the rat brain class A Ca channel sequence (with greater than 90% identity in the four repeated homology segments; see abstract by Starr et al.). However, homology between the class A and B Ca channel peptides differs significantly in two putative cytoplasmic domains. The first of these domains is located between homology repeats II and III (approx. 430 amino acids in rbB), while the second follows repeat IV (approx. 600 residues). In both the class A and class B peptides these two regions contain a number of potential cAMP-dependent phosphorylation sites. Studies are now underway to examine the temporal and spatial expression patterns of the rbB gene in the rat CNS, and to express a full-length rbB cDNA in a number of test systems.

486.7

ISOLATION OF cDNA CLONES FOR α1 SUBUNITS ISOFORMS OF THE L-TYPE CALCIUM CHANNEL FROM MOUSE BRAIN. W-J. Ma* and M. Uhler. Mental Health Research Institute, Univ. of Mich., Ann Arbor, MI 48109.

The α1 subunit is the major transmembrane component of the L-type calcium channel. Furthermore, it contains the binding site for dihydropyridines and has been proposed to form the ionic pore of the channel. In order to characterize the neural expression of the a1 subunit we have used a synthetic oligonucleotide complementary to the sixth transmembrane segment of the third repeat of the α1 subunit of the rabbit skeletal muscle calcium channel. cDNA clones related to the rabbit skeletal α1 sequence were isolated from mouse brain cDNA libraries. Two classes of cDNA clones were obtained. The first class of cDNA clones hybridizes to messenger RNAs of 8 and 14 kb in mouse brain and appear to code for the mouse cardiac form of the α1 subunit. This cDNA codes for a protein that is 98% identical in amino acid sequence to the rabbit cardiac α1 subunit. The second class of cDNA clones hybridize to mRNAs of 10 and 12 kb. This second type of cDNA codes for a protein highly homologous (75% identical) to the cardiac α1 subunit but contains many differences in nucleotide sequence from both the cardiac and skeletal sequences. These findings suggest that this class of cDNA results from transcription of a novel α1 subunit genomic sequence.

486.8

SINGLE CHANNEL PROPERTIES OF Ca CHANNELS EXPRESSED FROM RAT BRAIN mRNA IN *Xenopus* OOCYTES. Lin, J-W.and Llinás, R. Dept. Physiology and Biophysics, NYU Med. Ctr, NY, NY 10016.

Calcium channels expressed from rat brain mRNA in *Xenopus* oocytes have been shown to be different from L, N and T types in that this calcium current is activated by high voltages, with little inactivation, and is insensitive to dihydropyridine or ω-conotoxin (Leonard et al., J. Neurosci. 7, 875, 1987). We have shown: (1) this current can be blocked by spider toxin (Lin et al., PNAS, in press, 1990)(FTX) from *Agelenopsis aperta* (Llinas et al., PNAS. 86, 1689; Cherksey et al., Soc. Neurosci, abtr, 15, 652, 1989) and (2) the effect of the toxin is dependent upon the concentration of divalent cations. In order to further characterize this current, single channel recordings were obtained from on cell patches, using an intracellular electrode to monitor membrane potential. The patch was clamped at -100 mV and 40 msec pulses were used to activate voltage sensitive calcium channels. With 70 mM $BaCl_2$ and 1 μM TTX in the patch pipette, an inward unitary current of 0.58 pA was recorded at -20 mV and its amplitude decreased with further depolarizations. I-V plot of this unitary current, ranging from -40 mV to 0 mV, gives a slope conductance of 13 pS. In addition, in some patches, we observed a large unitary current with a slope conductance of 20 pS. The two unitary currents exhibited different sensitivities to depolarizations. For example, in one patch, the large unitary current was observed in 30% of the traces at 0 mV whereas the small unitary current in the same patch appeared in more than 65% of the traces at -20 mV. Both unitary currents appear throughout the duration of depolarizing pulses, suggesting a non-inactivating nature similar to the macroscopic calcium current recorded in this preparation. The effect of FTX on these channels are under investigation. (Supported by Fidia Research Foundation and NIH grants NS13742 to R. Llinas, GM26976 to B. Rudy).

486.9

EXPRESSION OF A RECOMBINANT OMEGA-CONOTOXIN IN YEAST. M.W. McLane*[1], R.A. Lampe[1], D. Shields*[2], A. Salama[1] and M.M.S. Lo[1]. [1]ICI Americas, Wilmington, DE 19897, and [2]Dept. of Developmental Biology and Cancer, Albert Einstein College of Medicine, Bronx, NY 10461.

Omega-conotoxins are potent blockers of N-Ca^{++} channels within various species. Several naturally occurring variants have been isolated from the venom of Conus snails. These complicated small peptides, which contain 24-29 residues, are highly homologous, and are predicted to have a rigid structure constrained by 3 intramolecular disulfide bridges. These peptides offer a substantial challenge for their correct processing, folding and secretion in yeast, which results in the production of a biologically active molecule. A synthetic oligonucleotide encoding a variant of omega-conotoxin was fused to the pro-alpha-factor sequence. Yeast strains, transformed with the recombinant pro-alpha-factor-toxin construct, produced a major low molecular weight peptide. The amino acid sequence is being determined on a reduced and carboxymethylated fraction. Disulfide bridge assignment will be determined in the native yeast product after purification by reverse phase HPLC. The biological activity of the recombinant conotoxin will be initially determined by a toxicity assay in goldfish, and further characterized by inhibition of ^{125}I-conotoxin (G6A) to rat brain membranes.

486.10

MULTIPLE BINDING SITES FOR 1,4-DIHYDROPYRIDINES ARE ASSOCIATED WITH SKELETAL MUSCLE CALCIUM CHANNELS. S.M.J.Dunn, C.Bladen* & R.P.Thuynsma*. Department of Pharmacology, University of Alberta, Edmonton, Alberta T6G 2H7.

The binding of (+)-[^{3}H]PN200-110, a calcium channel antagonist, to membrane preparations from rabbit skeletal muscle has been investigated in equilibrium and kinetic experiments. At equilibrium, binding is to an apparently homogeneous class of high affinity sites characterized by a K_d of 0.30 ± 0.05 nM (25 mM Hepes-Tris pH 7.4, 1 mM $CaCl_2$, 25°C). Measurements of the kinetics of association have suggested that complex formation is a simple bimolecular process. Under pseudo-first order conditions, the association reaction was monophasic, and the rate increased linearly with ligand concentration (0.1 - 2.0 nM). From these data, association and dissociation rate constants have been estimated to be 5.5 x 10^6 $M^{-1}s^{-1}$ and 0.0033 s^{-1} respectively. Direct measurements of the dissociation of (+)-[^{3}H]PN200-110 have, however, revealed a number of complexities. When dissociation was initiated by the addition of excess unlabelled (+)PN200-110, (±)nitrendipine or (±)Bay K8644, the dissociation rate increased with the concentration of competing ligand above 1 μM. The isomer, (-)PN200-110, at concentrations up to 30 μM, did not accelerate the dissociation rate. These results suggest the presence of an additional low affinity site (or sites) for 1,4-dihydropyridines (Kd ≈ 10 μM) which cannot be detected in direct binding studies. The low affinity site is stereoselective and its occupancy increases the rate of dissociation of [^{3}H]PN200-110 from its high affinity site. (Supported by NIH GM-42375.)

486.11

IDENTIFICATION OF RYANODINE BINDING PROTEINS IN THE AVIAN NERVOUS SYSTEM: LOCALIZATION IN PURKINJE CELLS DIFFERS FROM IP3 RECEPTORS. Mark H. Ellismanψ Thomas J. Deerinck*ψ Yannan Ouyang*ψ Claudia F. Beck*Σ Steven J. TanksleyΦ Philip D. Walton*Φ Judith A. Airey*Φ John L. Sutko*Φ.

ψNeurosciences, U.C. San Diego, La Jolla, Calif., 92093; Σ Biochemistry, Univ. of Idaho, Moscow, Idaho, 83843; Φ Pharmacology, Univ. of Nevada, Reno, Nevada, 89557.

We have identified ryanodine binding proteins in cells of the avian central nervous system. Monoclonal antibodies against avian skeletal muscle sarcoplasmic reticulum foot proteins were used to localize and isolate the brain proteins. Neuronal ryanodine binding protein-like immunoreactivity was found on components of the internal membrane system of cerebellar Purkinje neurons. The subcellular distribution of the immunoreactivity in these cells was established using laser confocal microscopy of thick sections, immuno-EM of peroxidase labelled semi-thick sections and by immuno-gold labelling of ultrathin cryosections. The ryanodine binding protein-like immunoreactivity was found within membrane systems of the perikaryon, the dendritic arbor and the axoplasm. Ryanodine binding proteins were not found in dendritic spines. Double immunolabeling experiments, comparing the distributions of the ryanodine binding protein to the IP3 receptor and to calbindin, revealed differences in cytoplasmic distributions. For example, the IP3 receptor immunoreactivity is prominent in the RER and dendritic spines (c.f. Mignery et al., Nature 342:192-195,1989) while the ryanodine binding protein appears absent in spines and is not distributed throughout the RER. Immunoprecipitation and [3H]epiryanodine binding experiments revealed that the cerebellar ryanodine binding proteins have a native molecular weight of ~2,000 kd and are composed of two high molecular weight (~500 kd) polypeptide subunits. A comparable protein having a single high molecular weight polypeptide subunit was observed in the remainder of the brain. If the ryanodine binding proteins in muscle and nerve are similar in function, then the neuronal proteins may participate in release calcium from intracellular stores that are distinct from those of the recently characterized IP3-activated calcium channel.

486.12

PHARMACOLOGICAL CHARACTERIZATION OF HIGH AFFINITY [^{3}H]RYANODINE BINDING SITES TO SUBCELLULAR FRACTIONS OF RAT BRAIN. R.A. PADUA, * J.D. GEIGER, W. WAN, * J.I. NAGY. Depts. Pharmacol. & Physiol., Univ. of Manitoba., Winnipeg., MB. R3E OW3 CANADA.

Ryanodine, at low nanomolar concentrations applied to neurons in vitro, inhibits the release of calcium from caffeine-sensitive intracellular sites that appear to be localized to endoplasmic reticulum. In analogy to muscle sarcoplasmic reticulum, this inhibition in neural tissues may be mediated through high-affinity ryanodine binding sites. To investigate whether specific [^{3}H]ryanodine binding sites are present on CNS tissues we conducted a series of studies using standard radioligand binding methods. Optimal binding was obtained at pH 8.0 in the presence of 550 μM ATP, 100 μM Ca^{2+}, and 1.0 M KCl. [^{3}H]Ryanodine binding was linear at membrane protein concentrations ranging from 0.02 to 0.13 mg. In association experiments, equilibrium was reached within 15 min at 37°C and levels of [^{3}H]ryanodine binding were stable for at least 2 hr. K_D values for P_1 (nuclear), P_2 (mitochondrial), and P_3 (microsomal) fractions derived from saturation experiments were comparable at about 5 nM. The number of [^{3}H]ryanodine binding sites in P_3 fractions was approximately 4-fold greater than that in P_1 or P_2 fractions. [^{3}H]Ryanodine may be binding to sites that mediate intracellular calcium release. (Supported by MRC of Canada).

486.13

CHARACTERIZATION OF A PUTATIVE INSECT CNS CALCIUM CHANNEL, USING THE PHENYLALKYLAMINE [^{3}H](±)VERAPAMIL. J.M. Skeer* and D.B. Sattelle. AFRC Laboratory of Molecular Signalling, Department of Zoology, Downing Street, Cambridge CB2 3EJ, U.K. Spon: Brain Research Association

Membranes prepared from the CNS of the cockroach, Periplaneta americana, have been found to contain a saturable, specific phenylalkylamine binding site, based on studies using [^{3}H](±)verapamil. Scatchard analysis has revealed a single population of binding sites with a dissociation constant (K_D) of 57 ± 5nM (mean ± SD, n=3) and a binding capacity (B_{max}) of 70 ± 20pmol per mg membrane protein (mean ± SD, n=3). Hill plots of the data yield a Hill coefficient of 0.98 ± 0.02 (mean ± SD, n=3) that does not depart significantly from unity, indicating the absence of co-operativity. Displacement studies using a range of putative calcium channel ligands reveal that the pharmacological profile of this [^{3}H](±)verapamil receptor in cockroach CNS membranes differs from that of the well-documented vertebrate phenylalkylamine binding receptor that is a component of an L-type calcium channel. In particular, the insect CNS phenylalkylamine receptor is dihydropyridine-insensitive, in contrast to the finding for the corresponding site in vertebrates which is dihydropyridine-sensitive. These data indicate the presence of a putative calcium channel in the insect CNS that is not readily classified using pharmacological criteria established for vertebrate tissues.

CATECHOLAMINES VI

487.1

IMMUNOHISTOCHEMICAL CHARACTERIZATION OF DISSOCIATED RAT LOCUS COERULEUS NEURONS IN CULTURE. C.G. Frondoza, J.-M. Fritschy, R. Grzanna, and M. Geffard. Johns Hopkins Univ., Depts. Immunology & Infectious Diseases and Neurosci., Baltimore, MD 21205, and University of Bordeaux, Lab. Immunol. and Neurobiol., 33076 Bordeaux, France.

Rat locus coeruleus (LC) neurons contain norepinephrine (NE) and several neuropeptides. To develop an experimental model system for studies of factors that may play a role in specifying transmitter phenotypes, we have grown LC neurons in culture following the protocol of Nakajima *et al.* In this study we identified by immunohistochemistry neurochemical markers of cultured LC neurons. The LC was isolated from 1-3 day old rats, dissociated and plated on a feeder layer of astrocytes. After 7-14 days incubation, culture plates were processed for immunohistochemistry using antibodies to glial fibrillary acidic protein, neuron specific enolase, tyrosine hydroxylase (TH), dopamine-ß-hydroxylase (DBH), NE, neuropeptide Y (NPY), serotonin and choline acetyl-transferase (ChAT). Between 50-70% of the neurons could be identified by TH immunohistochemistry as LC neurons. The morphologic appearance of these TH+ cells was very similar to that seen in the rat LC. Most TH+ cells were large, multipolar and formed extensive arrays of fine varicose processes. Most of the small TH+ cells were bipolar. All TH+ neurons also stained for DBH and NE. Many neurons in the culture stained for NPY and about half of those also stained for TH. In TH/NPY positive neurons the staining of the cytoplasm was consistently faint while their varicose processes were intensely stained. Neurons which contained only NPY had darkly stained cell bodies. Almost all TH positive neurons also contained serotonin, but none could be stained with ChAT. Dissociated LC neuron cultures should provide a valuable experimental model to identify and characterize factors that determine the neurotransmitter phenotype in these neurons. Support: MH 41977 and CA 45640.

487.2

DISTRIBUTION OF LOCUS COERULEUS NEURONS THAT PROJECT TO RAT VB THALAMUS AND BARRELFIELD CORTEX. D.W. Altman, C-S. Lin and B.D. Waterhouse. Dept. Physiol. and Biophys., Hahnemann Univ., Phila., PA. 19102

Previous studies from this laboratory have demonstrated a topographic relationship between projection neurons of the locus coeruleus (LC) and target regions of the neocortex and subcortical visual structures within the rat brain. The present investigation was conducted to examine the distribution of LC neurons that project to the ventrobasal (VB) nucleus of thalamus and the barrelfield region of the somatosensory cortex. An additional goal of the study was to determine if regions along the same sensory pathway receive collateral inputs from the same LC neuron. Long-Evans hooded rats (275-400 g) received unilateral pressure injections (1.0 ul) of various combinations of green and red fluorescent latex (rhodamine) microspheres into the barrelfield area of the somatosensory cortex and the VB nucleus. Coronal sections (80-100 um) through the LC were examined by fluorescence microscopy and the distribution of retrogradely labeled cells was recorded. LC neurons labeled from barrelfield injections were primarily located throughout the rostrocaudal dimension of the ipsilateral nucleus. By contrast, neurons projecting to VB thalamus were distributed bilaterally with a slight ipsilateral predominance. In the coronal plane, coeruleo-cortical projection neurons tended to be localized within the dorsal half of the LC, while cells projecting to VB thalamus had a tencency to be displaced more ventrally. Cells which were double-labeled were concentrated in the region of the ipsilateral nucleus where the subsets of cortical and thalamic projection neurons overlapped. In summary, the distribution of retrogradely labeled cells observed here suggests a rough topographic ordering of LC neurons with respect to somatosensory projection sites. Furthermore, the presence of double-labeled cells suggests that individual LC neurons may simultaneously influence the sequential processing of information at different levels within the afferent somatosensory pathway. (Supported by AFOSR 87-0138 and by an award from the Klingenstein Foundation to BDW)

487.3

NEURONS IN THE ROSTRAL MEDULLA PROJECT TO BOTH THE LOCUS COERULEUS (LC) AND THE NUCLEUS OF THE SOLITARY TRACT (NTS) IN THE RAT. E.J. Van Bockstaele[1,2], Yan Zhu[1*] and G. Aston-Jones[1]. [1]Dept. Mental Health Sciences, Hahnemann Univ., Phila., PA. 19102, [2] Dept. of Biology, New York University, N.Y., 10003.

Numerous anatomical and physiological studies have confirmed the existence of projections from the rostral ventrolateral medulla (RVLM; nuc. paragigantocellularis) and dorsomedial medulla (DMM; nuc. prepositus hypoglossi) to the LC. The functional significance of these circuits, however, remains unclear. To better understand their function, we have examined other connections of these LC afferents using double retrograde labeling techniques. Iontophoretic deposits of Fluoro-Gold (FG) were made into the LC and pressure injections of a complex of wheat germ agglutinin-conjugated apo (inactivated) horseradish peroxidase coupled to colloidal gold particles (WGA-apoHRP-Au) were placed into the NTS or into the ventrolateral portion of the periaqueductal gray (PAG). The silver intensification of the WGA-apoHRP-Au procedure did not appear to compromise the intensity or sensitivity of the fluorescent retrograde tracer, FG. The labels were identified within the same tissue section with fluorescent and light microscopy. Many doubly labeled neurons were seen in the RVLM following injections into the NTS and LC. Doubly labeled neurons were occasionally observed in the DMM immediately dorsal to the medial longitudinal fasciculus. In contrast, only a small number of doubly labeled neurons within the RVLM, and none within the DMM, were found following injections into the PAG and LC although singly labeled neurons for the two tracers were interdigitated. These results indicate that separate populations of neurons project to the LC and the PAG from RVLM and DMM. However, many RVLM neurons and some DMM neurons send collaterals to both the LC and the NTS. These results link the NTS and LC by their common afferents, indicating that these nuclei are closely coordinated in their functions. Supported by PHS grants NS24698 and DA06214.

487.4

THE EFFECTS OF VAGOTOMY ON THE FIRING PATTERNS IN LOCUS COERULEUS NEURONS. **C.L. Williams, E.J. Barea, S.E. Krahl, R.A. Jensen, and D.C. Smith. Department of Psychology, Southern Illinois University, Carbondale, IL 62901.**

This study investigated the role played by peripheral factors in the regulation of maintained discharge of locus coeruleus (LC) neurons. The response characteristics of these cells can be affected by systemic administration of substances which do not freely enter the brain. For instance, Holdefer and Jensen (Br. Res., 417:108, 1987) found a small decrease in spontaneous activity of LC cells following peripheral administration of 8.2 mg/kg 4-OH amphetamine, and an increase in maintained discharge with 10.0 mg/kg of epinephrine. The activity of these cells can also be affected by surgical interventions which remove some of the peripheral neural input to the CNS. For example, Svensson and Thoren (Br. Res., 172:174, 1979) showed that increasing blood volume alters the spontaneous activity of LC cells, and this effect was abolished by cervical vagotomy.

The present study was designed to examine the effects of vagotomy on the response characteristics and maintained discharge of neurons in the LC. Complete subdiaphragmatic vagotomy was performed on Sprague-Drawley rats 2 weeks prior to all testing. Electrophysiological recordings of single-units were measured in normal and vagotomized rats under urethane anesthesia with tungsten microelectrodes. In the vagotomized animals, we found a loss of spontaneous activity in some LC cells, as well as an altered response pattern to noxious external stimuli when compared to normal rats. These data suggest that peripheral factors transmitted to the brain via the vagus nerve play an important role in the modulation of the neuronal discharge patterns of CNS catecholamine systems. (Supported in part by NIMH 1 T32 MH-18882 to E.J.B.)

487.5

CHOLINERGIC MODULATION OF LOCUS COERULEUS: POSSIBLE ROLE IN CHOLINOLYTIC SEIZURES. M. El Etri, M.T. Shipley, M. Ennis, and W.T. Nickell. Depts. of Anatomy and Physiology, Univ. Cinti. Coll. Med., Cinti, OH. 45267.

Approximately 50% of rats given a systemic dose (0.7 LD_{50}) of the anticholinesterase soman develop convulsions. HPLC revealed a 50-90% reduction of forebrain norepinephrine (NE) in convulsing animals 50-120 min after AChE inhibition. Convulsing rats also had rapid (30-60 min) induction of c-fos in LC and layer of II piriform cortex, the most vulnerable site of soman-induced neuropathology. These results suggest that AChE inhibition induces a rapid elevation of LC neuronal activity and tonic NE release. This could result either because seizures increase LC activity or because AChE inhibition leads to hypercholinergic stimulation of LC neurons.

Microinjections of soman into LC rapidly and tonically (2-3 hrs) increased LC discharge rates 3-6 fold over baseline values. This activation was completely reversed by scopolamine (0.5 mg/kg, iv) but not mecamylamine (1.0 mg/kg, iv). Subsequent histochemical analysis revealed that AChE inhibition was confined to LC. In addition, c-fos expression was induced in LC and layer II of piriform cortex. These results suggest that LC neurons receive a tonic ACh input, that AChE inhibition leads to tonic ACh overstimulation of LC neurons causing tonic release of forebrain NE. AChE inhibition thus may cause combined ACh and NE hyperstimulation of cortical neurons. This may block afterhyperpolarization mechanisms leading to excessive cortical firing rates, seizures and cell death. (Supported by US Army DAMD17-86-C-6005 and PHS Grant NS24698).

487.6

ASYMMETRIC ORIENTATION OF LOCUS COERULEUS (LC) DENDRITES IN THE PERICOERULEAR REGION: BIOCYTIN-FILLED LC NEURONS IN VITRO. M.T. Shipley, G. Harris, J. Williams, E. Van Bockstaele, G. Aston-Jones, and M. Ennis. Dept. Anatomy, Univ. Cinti., Cinti, OH 45267; Hahnemann Univ, Philadelphia, PA 19102; Oregon Hlth Sci. Univ, Portland, OR.

We previously reported that LC neurons extend processes 400-500 um into two pericoerulear zones which we designated the rostromedial and caudal juxtaependymal pericoerulear regions. EM-immunocytochemical analysis demonstrated that virtually all of these processes are dendrites which are heavily targeted by several morphologically distinct classes of extrinsic synapses. These results suggest that the pericoerulear dendrites comprise a significant site for afferent synaptic regulation of LC neurons. However, it is not known whether the existence of extensive pericoerulear dendrites is a characteristic of many or only a subset of LC neurons. To address this issue we are analyzing the dendritic arbors of LC neurons impaled and filled with biocytin in horizontal brainstem slices.

Results to date suggest that: (1) most LC neurons have pericoerulear dendrites: (2) the longest of these dendrites extend into either the rostromedial or caudal juxtaependymal pericoerulear regions, or both: and (3) neurons in all parts of LC sampled exhibit this preferential dendritic organization.

These results suggest that many LC neurons receive significant afferent inputs on extranuclear dendrites which extend into two pericoerulear zones. EM double labeling methods are necessary to determine which afferents to pericoerulear regions terminate on LC dendrites, pericoerulear neurons or both. (PHS Grant NS24698).

487.7

EFFECTS OF LOCUS COERULEUS (LC) INACTIVATION ON ELECTROENCEPHALOGRAPHIC (EEG) ACTIVITY IN NEOCORTEX AND HIPPOCAMPUS. S.L. Foote, M.E. Page[1], C.W. Berridge, and R.J. Valentino[1]. Dept. Psychiatry (M-003), UCSD, La Jolla, CA 92093; (1) Dept. Mental Health Sci., Hahnemann Univ., Philadelphia, PA 19102.

The hypothesis that the noradrenergic neurons of the LC can influence forebrain EEG activity was investigated by inactivating these neurons in halothane-anesthetized rats while simultaneously recording neocortical (ECoG) and hippocampal (HEEG) EEG activity. LC discharge activity was reduced by infusing the alpha-2 noradrenergic agonist clonidine into LC. Unilateral infusions (240 ng/240nl/over 2 min), whose effects on LC activity were verified by microelectrode recordings, were followed by high-amplitude, low-frequency ECoG activity and mixed frequency HEEG activity. Bilateral clonidine infusions induced a nearly complete blockade of foot-pinch elicited EEG activation in this preparation. This blockade was potentiated by systemic or ICV clonidine. These results suggest that inhibition of LC activity may induce "hypersynchrony" in ECoG activity and may "disenable" or "block" HEEG theta activity under the conditions of this experiment. They also suggest that LC may influence forebrain EEG activity in the unanesthetized state. The present data complement our observations that LC activation in this preparation produces ECoG desynchronization and HEEG theta activity (Berridge et al., this meeting).

487.8

EFFECTS OF LOCUS COERULEUS ACTIVATION ON EEG ACTIVITY IN NEOCORTEX AND HIPPOCAMPUS. C. W. Berridge and S. L. Foote. Dept. Psychiatry, Univ. of Calif., San Diego, CA 92093.

The hypothesis that noradrenergic locus coeruleus (LC) neurons participate in forebrain activation as measured by EEG was examined. In halothane-anesthetized rats, small infusions (75-150 nl) of the cholinergic agonist, carbachol, were used to reversibly activate LC neurons while simultaneous recordings of LC neurons verified this activation. Power spectrum analyses of frontal neocortical and hippocampal EEG were performed. In 11 animals in which histologically verified peri-LC infusions activated the LC, the findings were: 1) LC activation was followed, within 2 to 30 sec, by a shift from low-frequency/high-amplitude to high-frequency/low-amplitude EEG activity in neocortex and the appearance of intense theta rhythm in the hippocampus; 2) In neocortex, absolute power of all frequency bands decreased; the largest decrease was observed in the 0.8-3.0 Hz band. Relative power increased in the 25.0-50.0 Hz and decreased in the 0.8-3.0 Hz bands; 3) Hippocampal relative power increased in the 3.0-7.0 Hz and decreased in the 0.8-3.0 Hz bands; 4) whether infusions were made lateral or medial to LC, forebrain EEG changes followed LC activation with similar latencies; 5) infusions that did not activate LC neurons (e.g., placed 1 mm dorsal or ventral to LC), had no effects on forebrain EEG activity; 6) EEG patterns returned to baseline with about the same time course as the recovery of LC activity (10-20 min). These observations indicate that LC activation is the crucial mediating event for these infusion-induced changes in forebrain EEG.

Supported by the U.S. Air Force (OSR) and the MacArthur Foundation.

487.9

STRESS-ELICITED ACTIVATION OF THE LOCUS COERULEUS (LC) BY NITROPRUSSIDE IS ASSOCIATED WITH EEG CORRELATES OF AROUSAL. M.E. Page and R.J. Valentino *Dept. of Mental Health* Sciences, Division of Behavioral Neurobiology, Hahnemann University, Phila, PA 19102.

Stressors have been shown to increase spontaneous discharge rates of LC neurons (Svensson, 1987). Recently, hemodynamic stress produced by nitroprusside infusion was found to increase LC discharge; this activation required endogenous corticotropin-releasing factor (CRF), suggesting that LC activation during stress is mediated by CRF (Valentino and Wehby, 1988). It was hypothesized that one function of LC activation is to increase or maintain arousal during hemodynamic stress. To test this hypothesis, the effects of nitroprusside on cortical and hippocampal EEG and LC spontaneous discharge were investigated in halothane-anesthetized rats. I.V. infusion of nitroprusside (10ug/30ul/min: 15 min) increased LC spontaneous discharge rate 31% ±13 (N=9). The increases in LC discharge were temporally correlated with EEG changes indicative of arousal, i.e., low amplitude, cortical desynchronization and onset of hippocampal theta rhythm. To elucidate the role of CRF in stress-induced EEG activation, a CRF antagonist, alpha-helical CRF_{9-41} was locally infused (100-150 ng) into LC prior to nitroprusside administration. Preliminary studies suggest that alpha-helical CRF_{9-41} (150 ng) may delay the onset, and shorten the duration of EEG activation. This work was supported by PHS Grants MH40008 and MH42796.

487.10

EFFECTS OF THE 5-HT REUPTAKE INHIBITOR FLUVOXAMINE ON ANXIETY INDUCED BY YOHIMBINE. A.W. Goddard, D.S.Charney, G.R. Heninger, S.W.Woods . Dept. of Psychiatry, Yale U. Sch. of Med., 34 Park St., New Haven, CT 06508.

The alpha-2 adrenergic receptor antagonist yohimbine (YOH) produces robust anxiogenic effects in panic disorder (PD) patients.This study investigated the effects of chronic treatment with the 5-HT reuptake inhibitor fluvoxamine (FLUV) on YOH-induced anxiety. METHODS: Fifteen patients with DSM III-R PD completed a double-blind placebo controlled study of FLUV. YOH (0.4 mg/kg IV) and placebo challenges were performed during a three week drug-free period and again after nine weeks of treatment. Within challenge measurements included visual analog scale (VAS) anxiety, DSM III-R somatic panic symptoms, blood pressure and heart rate, plasma cortisol (CORT), and 3-methoxy-4-hydroxy-phenethyleneglycol (MHPG). RESULTS: Six of 8 patients responded to FLUV, as compared to 2 of 7 receiving placebo. FLUV produced a greater reduction in the net YOH VAS anxiety response than placebo did, (with FLUV, 48.8±47.9 mm to 11.8±44.8, $p<.06$; with placebo, 41.9±20.9 to 40.7±26.3, NS; between FLUV and placebo, $p<.04$). FLUV but not placebo reduced somatic panic symptoms after YOH. Baseline and peak MHPG levels after YOH did not differ in FLUV compared to placebo treated patients. CORT levels in both groups rose after YOH but there was no significant effect of treatment in either group. Blood pressure and heart rate after YOH were not affected by FLUV treatment. DISCUSSION: Effective chronic treatment with FLUV appears to reduce YOH-induced anxiety and somatic symptoms. These results suggest that interactions between the 5-HT and noradrenergic systems may have a role in the therapeutic mechanism of the 5-HT reuptake inhibitors in PD.

487.11

PRIOR EXPOSURE TO CHRONIC STRESS RESULTS IN ENHANCED NOREPINEPHRINE SYNTHESIS IN RESPONSE TO A NOVEL STRESSOR. L.K. Nisenbaum, M.J. Zigmond, and E.D. Abercrombie. *Department of Behavioral Neuroscience and Center for Neuroscience, University of Pittsburgh, Pittsburgh, PA 15260*

We have shown that prior exposure to chronic cold stress results in enhanced hippocampal norepinephrine (NE) release in response to a novel stressor (Nisenbaum et al., Soc. Neurosci. Abstr., 15: 413, 1989). One factor that may contribute to this phenomenon is increased synthesis of neurotransmitter. To test this hypothesis, several measures of NE synthesis were carried out comparing naive and chronically cold stressed rats. Analysis of hippocampal tyrosine hydroxylase (TH) activity in vitro revealed no significant difference between the two groups, suggesting that chronic stress does not produce a detectable increase in the maximal amount of hippocampal TH enzyme activity. The synthesis of NE in vivo was assessed in two ways. First, the accumulation of 3,4-dihydroxyphenylalanine (DOPA) in hippocampus 30 min after administration of the aromatic amino acid decarboxylase inhibitor NSD-1015 (100 mg/kg, i.p.) was examined. Under basal conditions, DOPA accumulation was the same in both groups. In contrast, tail shock produced a greater increase in DOPA accumulation in chronically stressed animals (101 ± 15%) than in naive animals (45 ± 8%). Second, the level of extracellular 3,4-dihydroxyphenylacetic acid (DOPAC) was quantified in the hippocampus using in vivo microdialysis. The basal level of DOPAC did not differ between the two groups. In response to tail-shock stress, however, the increase in DOPAC efflux was larger in chronically stressed rats (136 ± 9%) than in naive animals (75 ± 8%). Thus, while the basal synthetic rate of NE does not differ between the two groups of animals, activation of TH by a novel stressor appears to be greater in chronically stressed rats than in controls.

487.12

CONTRIBUTION OF NORADRENERGIC AND DOPAMINERGIC NEURONS TO 3,4-DIHYDROXYPHENYLACETIC ACID (DOPAC) CONCENTRATIONS IN REGIONS OF THE HYPOTHALAMUS CONTAINING INCERTOHYPOTHALAMIC DOPAMINERGIC (IHDA) NEURONS. Y. Tian, K.J. Lookingland, and K.E. Moore, Dept. Pharmacol/Toxicol., Mich. State Univ., East Lansing, MI 48824

IHDA neurons have cell bodies (A_{13} group) in the medial zona incerta (MZI) and axons that project to the dorsomedial nucleus (DMN). The concentration of norepinephrine (NE) in these regions exceeds that of dopamine (DA), reflecting a higher density of NE nerve terminals; this can complicate interpretation of studies employing neurochemical techniques to estimate IHDA neuronal activity. The purpose of this study was to determine if concentrations of the DA metabolite DOPAC in MZI and DMN reflect the activity of IHDA neurons. Administration of apomorphine decreased, while haloperidol increased DOPAC concentrations in MZI and DMN. Injection of a NE neurotoxin (5-amino-2,4-dihydroxy-α-methylphenylethylamine) into the ventral NE bundle, reduced NE concentrations in the MZI and DMN by 80% while DA and DOPAC concentrations were unaltered. Activation of NE neurons with the α_2-adrenergic receptor antagonist idazoxan increased concentrations of 3-methoxy-4-hydroxyphenylethylene glycol and DOPAC in MZI and DMN in intact but not in ventral NE bundle-lesioned rats. These results reveal that DOPAC in MZI and DMN reflect the activity of IHDA neurons, but that when NE neurons are activated, significant amounts of DA are metabolized to DOPAC in NE terminals in these regions. (Supported by NIH Grant NS 15911.)

487.13

FUNCTIONAL AND NEUROCHEMICAL EVIDENCE THAT TUBEROHYPOPHYSIAL DOPAMINERGIC NEURONS IN THE RAT PROJECT FROM THE PERIVENTRICULAR NUCLEUS TO THE INTERMEDIATE LOBE OF THE PITUITARY. J.L. Goudreau, S.E. Lindley, K.J. Lookingland, K.E. Moore, Dept. of Pharmacol, Mich. State Univ., E. Lansing, MI 48824

Tuberoinfundibular dopaminergic neurons projecting to the median eminence tonically inhibit the release of prolactin, while tuberohypophysial dopaminergic (THDA) neurons projecting to the intermediate lobe (IL) of the pituitary inhibit the release of α-melanocyte stimulating hormone (αMSH). It was initially proposed (Björklund, et al., Brain Res. 51:171, 1973) that cell bodies of both of these neuronal systems in the rat were located in the arcuate nucleus (A_{12} cell group). Results of a more recent anatomical study (Kawano and Daikoku, J. Comp. Neurol. 265:242, 1987) suggest that THDA neurons originate from the A_{14} cell group in the periventricular nucleus. In the present study a Halasz knife cut in the ventral hypothalamus between A_{12} and A_{14} reduced by 50% the concentrations of dopamine (DA) and its metabolite, 3,4-dihydroxyphenylacetic acid (DOPAC) in IL, but had no effect on DA or DOPAC concentrations in the median eminence. Electrical stimulation of the periventricular nucleus increased the DOPAC/DA ratio in IL and reduced the secretion of αMSH; in the same animals the DOPAC/DA ratio in the median eminence and secretion of prolactin were unaltered. These results provide neurochemical and functional evidence that THDA neurons project from periventricular nucleus to the IL. (Supported by USPHS grant NS15911.)

487.14

D2 DOPAMINE AGONISTS ACUTELY INCREASE THE ACTIVITY OF TUBEROINFUNDIBULAR DOPAMINE NEURONS. S.A. Berry* and G.A. Gudelsky, Departments of Pharmacology and Psychiatry, Case Western Reserve University, Cleveland, OH 44106.

The activity of tuberoinfundibular dopamine (TIDA) neurons, in contrast to that of nigrostriatal neurons, is not acutely affected by typical dopamine antagonists, e.g., haloperidol, or agonists, e.g., apomorphine. However, we have reported that the activity of tuberoinfundibular dopamine (TIDA) neurons is acutely suppressed by D1 type dopamine agonists (Neurosci. Abstr. 15:1000, 1989). In the present study, we have examined the effects of the selective D2 agonists quinpirole and quinelorane on the activity of TIDA neurons, as judged from DOPAC concentrations and the in vivo accumulation of DOPA after decarboxylase inhibition in the median eminence. The accumulation of DOPA in the median eminence was dose-dependently increased by quinpirole (0.1-2.5 mg/kg, ip) and quinelorane (0.025 mg/kg, ip). Quinpirole and quinelorane also significantly increased the concentration of DOPAC in the median eminence. The activity of TIDA neurons was increased within 1 hr after the administration of quinpirole, and it remained elevated for 4 hrs. The stimulatory effect of quinpirole on TIDA neurons was completely antagonized by haloperidol (1 mg/kg, ip) but was unaffected by SCH 23390 (0.5 mg/kg, ip). It is concluded that D2 receptor stimulation results in an acute increase in the activity of TIDA neurons.

487.15

DOPAMINE NEURONS IN THE GUINEA PIG HYPOTHALAMUS: ELECTROPHYSIOLOGICAL PROPERTIES AND EFFECTS OF OPIOIDS. M.D. Loose, O.K. Ronnekleiv and M.J. Kelly. Dept. Physiology, Ore Hlth Sci Univ., Portland, OR 97201.

Dopamine-containing (DA) neurons in the arcuate nucleus of the guinea pig were recorded in hypothalamic slices. DA neurons, identified immunocytochemically by the presence of tyrosine hydroxylase, had a mean length to width profile of 14.9 ± 4.4 X 11.5 ± 3.1 μm (N = 14), a Na^+ action potential of short duration (0.8 ms) and an after-hyperpolarization of 6-9 mV with a decay half-time of ≈1.5 sec following induction of repetitive firing (20 to 50 Hz). DA cells exhibited a low threshold spike (LTS) which induced 1-4 Na^+ action potentials. The LTS was identified as an inward current which activated positive to -70 mV and was sensitive to high Mg^{2+}/low Ca^{2+} media but not to TTX. DA neurons also had a time-dependent inward current at potentials negative to -70 mV. Cs^+ blocked this conductance. The μ-opioid agonist, Tyr-D-Ala-Gly-MePhe-Gly-ol, hyperpolarized (14 ± 3 mV) DA neurons via induction of an outward current (93 ± 44 pA) which had a reversal potential similar to that of a selective potassium conductance. TTX did not block the opioid effects. Therefore, DA neurons of the arcuate nucleus differ in their intrinsic conductances and their responsiveness to opioids from other CNS DA neurons. Furthermore, opioid activation of a potassium conductance in DA neurons of the arcuate nucleus may underlie the effects of opioids on dopamine-mediated prolactin release.

487.16

CENTRAL D1 AND D2 DOPAMINE RECEPTORS STIMULATE HYPOTHALAMIC-PITUITARY-ADRENAL (HPA) ACTIVITY. B.Borowsky and C.M.Kuhn. Dept. of Pharmacology, Duke University Medical Center, Durham, NC 27707.

Non-selective and D2 selective DA agonists as well as cocaine and other inibitors of DA uptake stimulate the secretion of ACTH and corticosterone (CS) in rats. We now report that both D1 and D2 DA receptors contribute to this HPA stimulation, and that brain regions accessible to the third ventricle, such as the hypothalamus, may be involved in this response. Both the D1 selective agonist (±)SKF38393 (1 to 20 mg/kg, i.p.) and the D2 selective agonist quinpirole (0.1 to 3.0 mg/kg, i.p.) produced dose-dependent elevations in serum ACTH and CS. The HPA response to SKF38393 was attenuated by the D1 selective antagonist SCH23390 (0.25 mg/kg) while the HPA response to quinpirole was attenuated by the D2 selective antagonist (±)sulpiride (50 mg/kg). Administration of either SKF38393 or quinpirole (1 to 100 ug) to the third ventricle via chronic indwelling cannulae caused a dose-dependent elevation in ACTH secretion. Pretreatment with SCH23390 or sulpiride (i.p.) attenuated the ACTH response to i.c.v. SKF38393 or quinpirole, respectively. Similarly, administration of the selective DA uptake inhibitor GBR12909 (3 to 60 ug, i.c.v.) dose-dependently elevated serum ACTH and CS levels. These results suggest that DA terminals near the third ventricle play a role in the regulation of HPA activity through actions on both D1 and D2 DA receptors. In addition, the DA system mediating this endocrine response appears to be independent from forebrain DA systems mediating locomotor and rewarding responses. Supported by NIDA P50 DA05303-01A1.

488.1

EFFECT OF LONG TERM TREATMENT WITH PHORBOL ESTERS ON THE RELEASE OF NORADRENALINE FROM THE MOUSE ATRIA. S. Foucart*, I.F. Musgrave*, H. Majewski* and J. de Champlain. Department of Pharmacology, University of Melbourne, Parkville, Victoria, 3052, Australia.

The role of protein kinase C (PKC) in sympathetic neurotransmission was investigated by down regulation induced by long term exposure to phorbol esters. In the present study, mouse atria were incubated for 10 hours at 37°C in the presence of either phorbol myristate acetate (PMA, 1 μM) or phorbol dibutyrate (PDB, 1μM). The atria were then incubated with [^{3}H]-noradrenaline and following 60 min of washing, the atria were field stimulated at 5 Hz and the stimulation-induced (S-I) outflow of radioactivity was taken as an index of noradrenaline release. In control experiments, PMA (1 μM), PDB (1 μM) or a combination of 8-bromo cyclic AMP (90 μM, 8-Br) and isobutylmethylxanthine (100 μM, IBMX) significantly increased the S-I outflow to 177%, 230% and 169% of control respectively. Pretreatment with PMA or PDB completely abolished the enhancing effect of subsequent exposure to PMA and PDB suggesting down regulation of PKC. The effect of 8-Br + IBMX was significantly reduced by PMA pretreatment and abolished by PDB. The results observed with 8-Br + IBMX suggest that there is an interaction between PKC and protein kinase A. Moreover, since the pretreatment with PMA and PDB did not alter the absolute release of noradrenaline, it appears that protein kinase C is not implicitely involved in the noradrenaline release process.

488.2

IMMOBILIZATION STRESS INDUCES TRANSLOCATION OF PROTEIN KINASE C(PKC) IN RAT BRAIN. K.Yurko, H.Y. Wang and E.Friedman, Dept. of Psychiatry and Pharmacology, Medical College of PA., Philadelphia, PA. 19129.

PKC's role in mediating neurophysiological and behavioral events is expanding. Modulation of PKC activity was investigated with the paradigm of immobilization stress which induces both a physiologically and behaviorally "activated" state of the animal. Male Sprague-Dawley rats were subjected to immobilization stress for 2 hrs/d (acute:1 d stress;chronic:10 d stress) and sacrificed 24 hrs. after last stress session. The cytosol(c) to membrane(m) distribution of PKC activity in brain slices and synaptosomes in control animals was 60:40%. Cortical and hippocampal slices showed significant shifts in PKC activity from c to m in chronically, but not acutely stressed rats vs. controls. Hippocampal synaptosomes also showed significant PKC translocation from c to m under these conditions. In cortical synaptosomes significant PKC translocation from c to m was found in both acute and chronically stressed rats vs. controls. Responses to the phorbol ester, PMA(162nM) were significantly attenuated in both acute and chronically stressed rats vs. controls in all preparations used. PKC translocation induced by immobilization stress may serve to modulate neurotransmission involved in stress responses. The mechanism for this effect is currently unknown.

488.3

ROLE OF PROTEIN KINASE C (PKC) IN MEDIATION OF DYNORPHIN(1-8) (DYN) AND MET^5-ENKEPHALIN (MET) RELEASE INDUCED BY ANGIOTENSIN II (ANG), ARACHIDONIC ACID (AA) AND PGE_2 IN PRIMARY CULTURED BOVINE CHROMAFFIN CELLS. H. H. Suh, M. K. McMillian, P. Hudson* and J. Hong. LMIN, NIEHS/NIH, Research Triangle Park, NC 27709.

We have previously reported that 24 hr treatment of chromaffin cells with ANG, AA and PGE_2 caused an increase of MET secretion. In the present study, effects of ANG, AA and PGE_2 on the release of DYN were examinedin bovine chromaffin cells. MET and DYN contents were measured by radioimmunoassay. We found that long-term stimulation (24 hrs) of the chromaffin cells with ANG (20 nM), AA (100 μM) and PGE_2 (10 μM) caused increases in secretion of DYN as well as MET. The amount of DYN secreted from chromaffin cells was approximately 1000-fold less than MET. To examine possible involvement of PKC, effects of protein kinase inhibitors on increases in secretion of MET and DYN induced by ANG, AA and PGE_2 were studied. Staurosporin (10 nM), a protein kinase inhibitor which has a high affinity (Ki=0.7 nM) for PKC, almost completely inhibited increased release of MET and DYN induced by ANG, AA and PGE_2. However, an equal nanomolar concentration of K252a, a protein kinase inhibitor which has a low affinity (Ki=25 nM) for PKC, had no effect. Our results indicate that PKC appears to be involved in increases of secretion of MET and DYN induced by ANG, AA and PGE_2.

488.4

EFFECTS OF NOREPINEPHRINE ON PROTEIN KINASE C IN RAT CEREBRAL CORTEX. G.S. Dhillon, D.L. Yourick, R.D. Greenwald & J.L. Meyerhoff. Dept of Medical Neurosciences, Walter Reed Army Inst. of Research, Washington D.C. 20307-5100.

Biological effects of hormones and neurotransmitters are believed to be mediated through the activation of either cAMP or calcium-regulated cellular events. We have previously reported (Soc. Neurosci. Abstr. 15: 1321, 1989) the effects of a number of peptides with neuromodulatory properties on adenylate cyclase activity in cortex. In the present study we investigated the effects of norepinephrine (NE) on protein kinase C (PKC) in rat cerebral cortex.

Slices were prepared by the method of Maier and Rutledge (JPET 240:729-736, 1987). Slices were treated for 10 minutes and total cytoplasmic and membrane (solubilized) PKC activity was recovered by DEAE cellulose chromatography. Calcium and phospholipid-dependent PKC activity was determined by monitoring [^{32}p]-γ-ATP incorporated into histone H1 substrate by a slight modification of methods of Kikkawa, U. et al. (JBC 257:13341-13348, 1982) and Thomas, P. et al. (Methods in Enzymology, vol. 141:399-411, 1987). NE (100 uM) significantly increased PKC activity in membrane solubilized and, to a lesser degree, in the cytosolic fraction. These results suggest that PKC as well as adenylate cyclase, serves as a signal transduction mechanism for NE in cerebral cortex. Modulatory interactions may possibly exist between adenylate cyclase and protein kinase C activity.

488.5

NEUROTENSIN INCUBATION WITH RAT STRIATAL SLICES: EFFECTS ON CALCIUM/CALMODULIN-DEPENDENT PROTEIN PHOSPHORYLATION. J.W. Kasckow, S.T. Cain and C.B. Nemeroff. Duke Univ. Med. Ctr., Durham, North Carolina 27710

Neurotensin (NT) is an endogenous brain tridecapeptide which fulfills many of the requisite neurotransmitter criteria. Both high and low affinity NT receptors have been identified (J Neurochem 50:1026, 1988). To characterize the mechanism of NT signal transduction we have investigated the action of NT on calcium/calmodulin-dependent phosphorylation in rat striatal slices. Striatal slices were incubated with or without NT (5 or 50 nM) for 3, 10, 16 or 30 minutes. This was followed by in vitro phosphorylation, electrophoresis and autoradiography. NT altered the calcium/calmodulin-dependent phosphorylation of specific proteins. For example, following incubation with 5 nM NT, phosphorylation of the 62 KDa protein was decreased at 3 minutes and 10 minutes relative to control; at 16 minutes it was increased. In contrast, with 50 nM NT, 62 KDa protein phosphorylation was increased at 3 minutes, but decreased by 16 minutes. NT produced no changes in the phosphorylation of this protein in the presence of cAMP. This 62 KDa phosphoprotein is likely the β subunit of the calcium/calmodulin dependent protein kinase. These changes above may reflect the ability of NT to modulate calcium-mediated transduction at the 3rd messenger level.
(Supported by NARSAD, NSF BNS-8910032 and NIMH MH-39415.)

488.6

ABSENCE OF A CHANGE IN DARPP-32 PROTEIN OR mRNA IN NEONATAL-6-OHDA-LESIONED RATS. G.R. Breese, J.O'Callaghan, M. Ehrlich, H.E. Criswell, R.A. Mueller, and P. Greengard. UNC School of Medicine, Chapel Hill, NC; EPA, RTP, NC; and Rockefeller University, NY.

DARPP-32 is a protein that is enriched in neurons associated with DA_1-receptors. Neonatal-6-OHDA (N-6-OH) lesioned rats are known to develop sensitized DA_1-receptors after repeated treatment (i.e., primed) with a DA_1 agonist. The present investigations determined whether priming of DA_1-receptors or acute treatment with SKF-38393 would alter the content of DARPP-32 protein or its mRNA in N-6-OH-lesioned rats. Rats were sacrificed by either focused microwave irradiation or decapitation, respectively, for these determinations. DARRP-32 content in the striatum, measured by immunoassay, was not affected by any of the treatments when compared to the concentration in unlesioned rats. A change in DARPP-32 mRNA as measured by Northern blot analysis also was not apparent in N-6-OH lesioned rats after priming of DA_1-receptor responses. Studies are underway to determine if the content of phosphorylated DARPP-32 is altered in N-6-OH lesioned rats. (Supported by NS-21345, HD-23042, NS-06801, and MH-02714).

488.7

DOPAMINE STIMULATES THE PHOSPHORYLATION OF SPECIFIC PROTEINS IN PHEOCHROMOCYTOMA CELLS. L. Janocko and R.M. Lewis. Allegheny-Singer Res. Inst. and Dept. NACS, Univ. Pittsburgh, Pittsburgh, PA 15261.

We are developing a cell culture assay for studying the short- and long-term effects of dopamine on protein phosphorylation and gene expression in the nervous system. We chose DARPP-32 as a model protein because its phosphorylation is increased by dopamine *in vivo*, and because the rat and mouse cDNAs are available. We tested PC12 (rat adrenal pheochromocytoma) cells first because of their similarity to neuronal cells, and because DARPP-32 is found in adrenal medulla. Confluent PC12 cells were incubated with dopamine in RPMI medium supplemented with ascorbate. The cells were collected, rapidly frozen and homogenized on ice. Half of each sample was incubated for 30' at 30°C to allow endogenous phosphatases to remove all phosphate groups, and thereby permit measurement of total phosphorylatable sites. Both sample halves then were quenched with zinc acetate, dissolved in citric acid, neutralized, phosphorylated by exogenous cAMP-dependent protein kinase and [γ-^{32}P]-ATP, resolved by SDS/PAGE and autoradiographed. A band at M_r~32K was phosphorylated in a dose and time dependent manner. The phosphorylation of numerous other bands was not affected. We currently are attempting to confirm that the band is DARPP-32, and to determine whether the effect is D1, D2 or autoreceptor specific.

488.8

POTENTIATION OF ENDOTHELIN- AND ATP-INDUCED PHOSPHOINOSITIDE TURNOVER BY CALCIUM IONOPHORES IN C_6-GLIOMA CELLS. W.-W. Lin, C.Y. Lee* and D.-M. Chuang. Biological Psychiatry Branch, NIMH, Bethesda, MD 20892 and Dept. of Pharmacology, National Taiwan University, Taipei, Taiwan

Endothelin-1 (ET) and ATP induce a robust increase of phosphoinositide (PI) metabolism in C_6 glioma cells and primary cultures of cerebellar astrocytes. In this study, we investigated interaction between Ca^{2+} ionophore, A23187 and ET- and ATP-induced ^{3}H-inositol monophosphate (^{3}H-IP$_1$) in these cells prelabeled with ^{3}H-myo-inositol. In C_6 glioma, A23187 dose-dependently potentiated the responses to ET and ATP with an EC_{50} of about 0.3 μM. The maximal stimulation of PI turnover induced by ET (30 nM) and ATP (100 μM) was increased from 23 to 42-fold and from 8 to 19-fold, respectively, by the presence of 10 μM A23187, which alone induced a 3.5-fold increase of ^{3}H-IP$_1$ accumulation. The EC_{50} values of ET (2 nM) and ATP (84 μM) were unchanged by this Ca^{2+} ionophore. Ionomycin also potentiated the efficacy of ET-induced PI hydrolysis in concentration range of 10^{-8}-10^{-6} M. In contrast to C_6-glioma, in cultured cerebellar astrocytes A23187 failed to induce synergistic effects on PI metabolism induced by ET, ATP, norepinephrine, angiotensin, bradykinin, and neurotensin, although the ionophore alone induced a more than 4-fold increase in ^{3}H-IP$_1$ accumulation. These results indicate that entry of Ca^{2+} has an important role in amplifying PI turnover stimulated by ET and ATP in C_6-glioma cells but not in cerebellar astrocytes.

488.9

WITHDRAWN

488.10

INHIBITORS OF PROTEIN KINASES DECREASE PEPTIDE SECRETION IN APLYSIA BAG CELL NEURONS. K.J. Loechner, J. Mattessich, and L.K. Kaczmarek. Dept. of Pharmacology, Yale Univ. Sch. Med., New Haven, CT 06510.

The bag cell neurons (BCNs) provide a model system for the study of neuropeptide release and its regulation by second messengers. Following electrical stimulation, the BCNs fire repetitively (afterdischarge) and release several neuroactive peptides including egg-laying hormone (ELH). During the afterdischarge, cAMP levels increase and phosphoinositide (PI) hydrolysis is stimulated leading to an activation of the kinases PKA and PKC. We have tested the effects of H8 and H7, two kinase inhibitors that act in intact cells with preferential specificity for PKA and PKC respectively, on the release of ELH. BCNs in intact ganglia were arterially perfused with artificial seawater (ASW), H8 (100μM) or H7 (50-100μM) for 30 min prior to stimulation of an afterdischarge. The surrounding medium was exchanged at 5 min intervals throughout the afterdischarge and the released material analyzed using HPLC. Release at each 5 min interval as well as total ELH release during the entire afterdischarge were reduced in both H8 (n=6) and H7 (n=10) as compared to ASW controls (n=8). These results were found with afterdischarges of various durations (range: 10-35 min). In sum, these data suggest that both PKA and PKC may modulate the amount of ELH released during an afterdischarge.

488.11

CORRELATION OF PHORBOL ESTER-INHIBITED PHOSPHOINOSITIDE METABOLISM WITH PROTEIN KINASE C-MEDIATED PHOSPHORYLATION OF RAT HIPPOCAMPAL MEMBRANE PROTEINS. L. M. Shaffer, Y. F. Han, and L. A. Dokas#. Departments of Biochemistry and Neurology#, Medical College of Ohio, Toledo, OH 43699.

In the brain, acetylcholine stimulates the breakdown of phosphatidylinositol 4,5 bisphosphate (PIP_2) to inositol triphosphate and diacylglycerol (DG), which activates protein kinase C (PKC). Active phorbol esters, which have been shown to block cholinergic stimulation of PIP_2 breakdown and which stimulate PKC, were used to identify membrane-associated protein substrates of this enzyme. When rat hippocampal slices were incubated with 10 μM 12-O-tetradecanoyl-phorbol-13-acetate (TPA), there was inhibition of cholinergic stimulation of PIP_2 breakdown, correlated with altered phosphorylation of two membrane proteins. Using 2-dimensional gel electrophoresis, post-hoc phosphorylation assays and western blotting, one protein was identified as B-50/GAP-43 (M_r~48,000, pI~4.5), a protein which may be involved in feedback inhibition of the PIP_2 second messenger cycle. The second protein responsive to TPA appears as a doublet on SDS-PAGE with a M_r of about 74,000 and a major component with a pI of about 6.9. Increased phosphorylation of this protein is the more prominent effect following incubation of hippocampal slices with $^{32}P_i$ and TPA. Further characterization of this protein is currently under way. Supported by grants from NIH (NS 23598) and the Ohio Department of Aging.

488.12

PROTEIN KINASE C AND CALCIUM/CALMODULIN KINASE II-STIMULATED PROTEIN PHOSPHORYLATION IN THE RODENT ANTERIOR PITUITARY J.C. Pryor & F. Sulser, Depts. of Psychiatry & Pharmacology, Vanderbilt Univ. Sch of Med., Nashville, TN 37232

Protein phosphorylation is recognized as a primary regulator of cell metabolism, including stimulated-secretion of hormones. In the past we have demonstrated the presence of cAMP-, calcium/phospholipid- and calcium/calmodulin-stimulated protein phosphorylation of endogenous anterior pituitary protein substrates in crude homogenates of the rat anterior pituitary. We have extended these findings to investigate the presence of endogenous anterior pituitary phosphoproteins in crude sucellular fractions of the rat anterior pituitary.

Normal male rats were killed by decapitation and the anterior pituitaries were removed and homogenized in ice cold buffer. The homogenates were centrifuged at 100,000x g for 1 hour, and the supernatant removed and designated the cytosolic fraction. The pellet was resuspended in the same volume of buffer and designated the particulate fraction of the tissue specimen. Protein phosphorylation was performed in the presence and absence of calmodulin (5 μg/ml), the calcium calmodulin kinase II inhibitor mastoparan (3 μM), phosphatidyl serine (35 μg/ml), and calcium (1mM). The reaction was carried out for 60 seconds. The samples were boiled and electrophoresed on 10% acrylamide SDS-PAGE gels. Autoradiographs of the gels were prepared and analyzed by scanning densitometry.

Proteins with molecular weights of 85.5 and 53.2 kD were identified in the cytosolic fraction to be phosphorylated in the presence of calmodulin (putative calcium/calmodulin kinase II activity), and those with molecular weights of 74.3 and 17.4 kD were identified in the cytosolic fraction to the phosphorylated by protein kinase C. Those with molecular weights of 23.3 kD, 19.7 kD, and 14.1 kD were identified in the particulate fraction to be phosphorylated by calcium/calmodulin kinase II, and those of 92.1 & 59.0 kD were phosphorylated by protein kinase C.
(Supported by USPHS grants MH-18921 & MH-29228))

488.13

PROTEIN KINASE C ISOFORMS DISTINGUISH MAJOR CELL TYPES IN RAT HIPPOCAMPUS. J.F. McGinty, W.T. Bohler*, M. Couce* and K. Ways*. Depts of Anatomy and Cell Biology and [1]Medicine, East Carolina University School of Medicine, Greenville, NC 27858.

Protein kinase C (PKC) types II/III (alpha/beta) immunoreactivity (IR) has been reported to be present in putative hilar basket cells and pyramidal cells of the hippocampal formation (Stichel and Singer Exp. Brain Res. 72:443, 1988). The present study investigated the distribution of PKC alpha, beta, and epsilon immunoreactivity (IR) and mRNA in the hippocampus using selective antisera and oligonucleotide probes in immunocyto- chemistry (ICC) and in situ hybridization histochemistry (ISHH) respectively. PKC antisera were raised against synthetic peptides with non-overlapping sequences and were found to be specific for each isoform by Western blot analysis. ICC was performed with standard avidin-biotin-peroxidase method. PKC alpha antisera preferentially stained two sets of neurons; hilar neurons shaped like basket cells subjacent to the granule cell layer displayed homogeneous cytoplasmic IR whereas CA3 pyramidal cells displayed a punctate reaction product (Stichel & Singer's type 1 and 2 cells). The neuropil in stratum radiatum and stratum oriens was also labeled in the terminal distribution pattern of Schaffer collaterals. PKC beta antisera stained CA3 pyramidal cells most intensely with a punctate distribution of IR. PKC epsilon antisera stained dentate granule cells and mossy fibers most intensely. Absorption controls demonstrated the specificity of each antiserum. ISHH substantiated the distributions revealed by ICC: PKC alpha mRNA was densest in the CA3 pyramidal cell layer although the CA1 pyramidal cell layer contained a less intense signal. The opposite distribution was true for PKC beta mRNA whereas PKC epsilon was most intense in the granule cell layer. Thus, different isoforms of PKC are preferentially expressed by the major cell types in the hippocampal formation. The regulation of expression of these different isoforms is under investigation. **Supported by DA 03982.**

488.14

PROTEIN KINASE C α-SUBSPECIES IS PRESENT IN GABAERGIC NEURONS OF THE MONKEY FRONTAL CORTEX. N. Saito, T. Tsujino*, S. Tominaga*, T. Oishi†, K. Kubota†, and C. Tanaka*. Dept. of Pharmacol., Kobe. Univ. Sch. of Med., Kobe 650, †Dept of Neurophysiol. Primate Res. Inst., Kyoto Univ. Inuyama, Aichi 484, Japan.

Protein kinase C (PKC) has been demonstrated to be a large family consisting of more than four PKC-subspecies (α, βI, βII and γ) by molecular cloning studies and PKC acts as a key enzyme in various neuronal signal transduction. We examined immmunocytochemically the distribution of each PKC subspecies in the frontal cortex of 3 monkeys (macaca fuscata) and Wistar rats. Detailed techniques were described elsewhere (J. Neurosci. 10, 870, 1990). Cells in layer I were stained by α-PKC but not by other subspecies in monkey, while the α-PKC-immunoreactive cells in layer I were not found in rat. The stained cells were oval or round in shape, 10-15 μm in size,and occationally stained with 1-3 proximal dedrites. Distributions and numbers of the staind cells in different cortical areas (Brodmann's areas 4, 6, 8, and 46) were comparable to those stained by GABA immunocytochemistry, indicating that almost of all GABA neurons in layer I are immunoreactive for α-PKC. Further, cells of similar shape and size were found in layers II-VI, being the most frequent in layer II and then in superficial part of layer III. These stained cells were also similar to GABA-immunoreactive cells. It appears that GABAergic neurons in the monkey frontal cortex contain α-PKC, suggesting that phosphorylation by α-PKC within GABAergic neuron is resposible for the frontal cortical functions of primates.

488.15

AMINO ACID ANALYSIS AND SEQUENCING OF RAT BRAIN PROTEIN PHOSPHATASE 2A. G.N. Barnes, T.C. Vanaman* and J.T. Slevin. Veterans Administration & U.Kentucky Med Ctrs, Lexington, KY 40536

Protein phosphatase 2A [pp2A], identified in rat hippocampus as a histone protein phosphatase activity, was totally inhibited by 1 nM Okadaic acid. Substrate specificity studies suggested that pp2A regulates autophosphorylated Ca^{+2} and cAMP-dependent protein kinases. With neuronal depolarization, pp2A activity redistributed from cytosol to synaptic plasma membranes. Purified rat brain pp2A is a trimer of 63, 55 and 38 kDa polypeptides. Amino acid (AA) analysis of the 63 and 38 kDa subunits revealed a composition nearly identical to the 65 and 38 kDa subunits, respectively, of pig kidney and rabbit skeletal muscle pp2A. Sequencing & AA analysis of 2 of 6 isolated CNBr fragments of the 38 kDa subunit suggest it is highly homologous to published sequences of the pp2A catalytic subunit. Sequencing & analysis of a 17 kDa CNBr fragment & a 12 kDa trypsin fragment of the neuronal 63 kDa subunit suggest its primary structure is highly homologous to the α isoform of the 65 kDa subunit from pig kidney and HeLa cells. Supported by V.A.Medical Research Funds.

EXCITATORY AMINO ACIDS: NON-NMDA RECEPTORS

489.1

STEREOSELECTIVITY AND MODE OF INHIBITION OF PHOSPHOINOSITIDE-COUPLED EXCITATORY AMINO ACID RECEPTORS BY 2-AMINO-3-PHOSPHONOPROPIONIC ACID B.G. Johnson, D.D. Schoepp, E.C.R. Smith*, and L.A. McQuaid, Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN 46285.

D,L-2-amino-3-phosphonopropionic acid (AP3), a phosphonate-substituted derivative of aspartic acid, has been shown to be an inhibitor of excitatory amino acid-stimulated phosphoinositide hydrolysis in rat brain slices. In this study, the enantiomers of AP3 were synthesized and used to further characterize the stereoselectivity and mechanism of interaction of this compound for inhibiting phosphoinositide-coupled (metabotropic) excitatory amino acid receptors. L-AP3 was 3-5 times more potent than D-AP3 as an inhibitor of ibotenate-stimulated ^{3}H-inositol monophosphate formation in slices of the rat hippocampus or quisqualate-stimulated ^{3}H-inositol monophosphate formation in neonatal rat cerebral cortical slices. Carbachol-stimulated phosphoinositide hydrolysis was not inhibited by L-AP3, and L-AP3 had no appreciable affinity for ionotropic excitatory amino acid receptors at concentrations required to inhibit metabotropic excitatory amino acid responses. The inhibitory effects of L-AP3 or L-2-amino-4-phosphonobutyric acid on phosphoinositide hydrolysis were non-competitive since they could not be surmounted by increasing concentrations of ibotenate or quisqualate. L-AP3 inhibition also could not be prevented by washing the tissue prior to incubating with ibotenate. Although L-AP3 is a stereoselective inhibitor of metabotropic excitatory amino acid receptors with little affinity for ionotropic receptors, the site at which it acts to inhibit metabotropic excitatory amino acid receptors remains to be determined.

489.2

PHARMACOLOGICAL BLOCKADE OF OSCILLATORY CURRENTS INDUCED BY *TRANS*-ACPD AND QUISQUALATE IN RAT BRAIN mRNA-INJECTED *XENOPUS* OOCYTES. G.B. Watson, D.T. Monaghan[1] and T.H. Lanthorn. CNS Diseases Research, G.D. Searle & Co., Skokie, IL 60077 and [1]Dept. of Pharmacology, University of Nebraska, Omaha NE 68105.

It has been suggested that *trans*-ACPD may be a selective agonist for the metabotropic quisqualate-sensitive receptor. In *Xenopus* oocytes injected with rat cortex mRNA, quisqualate induced both smooth and oscillatory currents indicating that it acts at more than one receptor. *Trans*-ACPD induced only oscillatory currents suggesting that it selectively activates the metabotropic receptor. The threshold concentration for *trans*-ACPD was approximately 30 μM. Oscillatory currents induced by both quisqualate and *trans*-ACPD were blocked by intracellular injection of EGTA. These oscillatory currents were also blocked by the application of 30 μM NMDA plus 10 μM glycine but not by NMDA alone. Depolarizing current injection did not mimic the effect of NMDA plus glycine. Oscillatory currents which had been blocked by EGTA could occur when NMDA plus glycine was co-applied with *trans*-ACPD. Smooth currents induced by quisqualate were unaffected by either EGTA or NMDA plus glycine. Oscillatory currents were not blocked by the non-selective acidic amino acid antagonist DNQX (10 μM).

489.3

A NEW POTENT METABOTROPIC GLUTAMATE RECEPTOR AGONIST. M. Ishida, H. Akagi[+], Y. Shimada* and H. Shinozaki. The Tokyo Metro. Inst. of Med. Sci., Tokyo 113, and [+]Mitsubishi Kasei Inst. of Life Sci., Tokyo 194, Japan.

α-(Carboxycyclopropyl)glycine(CCG) is a conformationally restricted analogue of glutamate and has eight stereoisomers theoretically. The (2S,3S,4S)isomer of CCG (L-CCG-I) caused a marked depolarization due to activation of non-NMDA, non-kainate or non-AMPA type receptors. The development of this new and potent agonist opens the possibility for extensive studies of the glutamate receptor subtypes. Peak amplitudes of responses to a possible metabotropic agonist, trans-ACPD, and L-CCG-I were significantly decreased when the temperature was decreased, while those of other excitatory amino acids was markedly increased, suggesting that the intracellular enzyme systems were related to the depolarization induced by L-CCG-I. Application of L-CCG-I induced the oscillatory responses in the Xenopus oocyte with injection of mRNA from rat brain, suggesting that L-CCG-I is a metabotropic receptor agonist. L-CCG-I is more potent than trans-ACPD in causing a depolarization in the rat isolated spinal cord. L-CCG-I depressed monosynaptic reflexes of the rat isolated spinal cord in considerably low concentrations. At the moment, it is not clear that this depression of monosynaptic reflexes is through activation of metabotropic receptors.

489.4

SENSITIZATION TO L-2-AMINO-4-PHOSPHONOBUTANOIC ACID (L-AP4) IS INDUCED BY AGONISTS SELECTIVE FOR PHOSPHOINOSITIDE-LINKED RECEPTORS. E.R. Whittemore and C.W. Cotman, Dept. of Psychobiology, Univ. of Calif., Irvine, CA 92717

Quisqualic acid (QA) sensitizes neurons to depolarization by D- and L-2-amino-4-phosphonobutanoic acid (AP4). This 30- to 100-fold sensitization persists for hours, and is induced through the interaction of QA with a receptor distinct from the QA/AMPA receptor ionophore. Only QA has been shown to induce this 'QUIS-effect'.

In order to test whether the QUIS-effect is induced via phosphoinositide (PI) - linked receptors, we assayed compounds reported to stimulate PI turnover for the ability to induce sensitization to L-AP4. Extracellular field potentials were recorded from the CA1 region of rat hippocampal slices. IC_{50} values for L-AP4 were obtained before and after exposure of slices to test compounds. Sensitization to L-AP4 was induced by trans-1-aminocyclopentane-1,3-dicarboxylic acid (trans-ACPD)(100 μM), ibotenic acid (100 μM, in the presence of 1 mM D-2-amino-5-phosphonopentanoic acid) and carbachol (500 μM). These compounds and concentrations are reported to stimulate phosphoinositide (PI) turnover in biochemical assays. However, the sensitization induced by these compounds was less (2- to 5-fold) than by QA (>30-fold), and reversed much more rapidly. For example, a 4 minute exposure to 100 μM trans-ACPD shifts the IC_{50} for L-AP4 from the pre-exposure value of 1800 μM to approximately 500 μM. In contrast, 16 μM QA shifts the IC_{50} for L-AP4 to 55 μM. These data suggest that the QUIS-effect may be induced in part via a PI-linked mechanism, and may represent a long-term physiological response to PI turnover in the CNS.

489.5

Effects of Quisqualate on Cytosolic Free Calcium in Single Cultured Rat Cerebellar Granule Cells. A.J. Irving*, G.L. Collingridge,*, L. Haynes,* & J.G. Schofield * (SPON: Brain Research Association) Departments of Biochemistry, Pharmacology and Zoology, University of Bristol, Bristol, BS8 1TD, UK.

Quisqualate can mobilize intracellular free calcium ([Ca]i) via a receptor that is linked to phospholipase C. This "metabotropic" receptor is believed to be present on cerebellar granule cells. We have studied the relationship between activation of this receptor and [Ca]i by obtaining simultaneous measurements from several individual granule cells within a field. Cells were loaded with FURA-2-AM and imaged using an intensified CCD camera linked to a Joyce-Loebl magical system.

In Ca free medium, 1 uM quisqualate caused a rapid transient elevation of [Ca]i. This effect declined following repeated applications but could be recovered following exposure to 1 mM [Ca]o. Reproducible transient responses could be obtained by applications of 1 uM quisqualate in the presence of 1 mM [Ca]o and 20-40 uM CNQX. In some cells, these responses were reversibly antagonised by 1 mM DL-AP3. These results are consistent with the notion that granule cells contain a Ca mobilising, quisqualate-activated receptor.

489.6

LACK OF EFFECT OF NBQX ON QUISQUALATE STIMULATED INOSITOL PHOSPHOLIPID HYDROLYSIS. P.D. Suzdak, A.J. Hansen, M.J. Sheardown, P. Jacobsen and T. Honoré. Novo Nordisk CNS Division, Sydmarken 5, DK-2860 Soeborg, Denmark.

2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo(f)quinoxaline (NBQX) is a potent and selective inhibitor of binding to the ionotropic quisqualate receptor (IC_{50} for inhibition of ^{3}H-AMPA binding = 150 nM). NBQX protects against global ischemia when administered up to 6 hours after reperfusion. The present report examines the effect of NBQX on the quisqualate receptor, coupled to inositol phospholipid hydrolysis (IPH). The effect of excitatory amino acids on IPH (as measured by the accumulation of ^{3}H-inositol monophosphate in the presence of LiCl) in cerebellar granule cells in culture from 7-day-old mice was examined. Quisqualate (0.1 - 300 μM), ibotenic acid (1 - 300 μM) and glutamate (10 - 300 μM) stimulated IPH in a dose-dependent manner. The ability of quisqualate to stimulate IPH was unaffected by CNQX (30 μM), whereas APV (1 mM) blocked the increase in inositol phospholipid hydrolysis produced by glutamate. NBQX (0.1 - 300 μM) was without effect on the basal levels of IPH. Furthermore, NBQX (0.1 - 300 μM) did not alter the stimulation of IPH produced by quisqualate (100 μM). NBQX (30 μM) also had no effect on the ED_{50} value for quisqualate stimulated IPH. The effect of NBQX in ibotenic acid and kainic acid stimulated IPH will also be discussed. These data suggest that the neuroprotectant effect of NBQX is mediated via the ionotropic, and not the metabotropic, quisqualate receptor.

489.7

CNQX AND APV INSENSITIVE GLUTAMATE RESPONSES IN RAT SUBSTANTIA GELATINOSA NEURONS. M. Yoshimura & T. M. Jessell, Center for Neurobiology and Howard Hughes Medical Institute, Columbia University, New York, NY. 10032

We have examined the pharmacological properties of l-glutamate evoked responses and primary afferent-evoked epsps in substantia gelatinosa (s.g) neurons using intracellular and whole cell voltage clamp recording from slices of adult rat spinal cord which retain dorsal root inputs. In addition, recordings were obtained from acutely isolated dorsal horn neurons from adult rats.

Stimulation of primary afferent evoked monosynaptic fast epsps in more than 70 % of s.g neurons. These epsps were markedly depressed by amino acid receptor antagonists, kynurenate and CNQX supporting the idea that glutamate or related amino acid is the transmitter mediating fast epsps. Bath applied glutamate produced a depolarization and inward current in a dose-dependent manner. The glutamate-evoked response was reduced in amplitude by only 20 % in the presence of non-NMDA receptor antagonist CNQX (5 uM). The CNQX-resistant component of the glutamate-evoked response did not result from activation of NMDA receptor.

We also examined glutamate-evoked currents in neurons isolated from the superficial dorsal horn of adult rat spinal cord. In about 60 % of neurons, inward currents evoked by glutamate, quisqualate (QA) and kainate (KA) were almost completely eliminated by CNQX (10 uM). However, in 40 % of neurons, CNQX depressed glutamate response by only about 30 %, even though QA and KA responses in the same neuron were depressed by more than 90 %. The CNQX-resistant glutamate response was not blocked by APV. These observations support the idea that a subset of dorsal horn neurons express two distinct non-NMDA receptors with differing sensitivities to CNQX.

489.8

NBQX, (2,3-DIHYDROXY-6-NITRO-7-SULFAMOYL-BENZO (f)QUINOXALINE): ANTICONVULSANT EFFECTS IN MICE. M. D. B. Swedberg, P. Jacobsen * and T. Honoré *. Novo Nordisk, Ferrosan CNS Division, Sydmarken 5, DK-2860 Soeborg, Denmark.

NBQX, an analog of the quinoxalinedione antagonists to non-NMDA glutamate receptors, potently and selectively inhibits AMPA receptor binding with no activity at NMDA or glycine sites, and protects against cerebral damage after global ischemia (1). The anticonvulsant effects of NBQX were evaluated by the intraperitoneal (ip) and the intraveneous (iv) routes against audiogenic seizures in DBA/2 mice, and against seizures induced by 150 mg/kg DMCM (ip; 30 min) in NMRI mice, at 5, 15 and 30 min prior to test. Sedation was assessed in a rotarod apparatus.

In DBA/2 mice NBQX was 5-17 (ip) and 2-3 (iv) times more potent as an anticonvulsant, ED50's (mg/kg) being 13.0, 8.2 and 3.5 (ip) and 1.9, 4.5 and 15.5 (iv) at 5, 15 and 30 min, respectively, than in producing ataxia. Against DMCM induced seizures, NBQX was ineffective at doses up to 30 mg/kg (ip), or ataxic at doses equal to, or lower than, the anticonvulsant doses (iv).

NBQX shows anticonvulsant effects in mice probably thru suppression of the activity of the non-NMDA excitatory amino acid quisqualate.

(1) Sheardown et al., Science 247:571-574,1990.

489.9

QUISQUALATE RECEPTOR ANTAGONISTS IN THE SUB-PALLIDUM (SP) DECREASE THE HYPERMOTILITY RESPONSE TO AMPHETAMINE. D.L. Willins*, D.E. Supko*, R.A. Hill*, L.J. Wallace*, D.D. Miller* and N.J.Uretsky. College of Pharmacy, The Ohio State University, Columbus, OH 43210.

Previous studies suggest that quisqualate (QUIS) receptors in the SP are involved in the stimulation of locomotion (LMA). AMPA, a QUIS agonist, upon injection into the SP, stimulates LMA. This effect is antagonized by the non-NMDA receptor antagonist DNQX and GAMS. We have recently found that both GAMS and DNQX can inhibit the hypermotility response to systemically administered amphetamine (AMPH). In order to determine whether the inhibitory effects of DNQX are mediated through the blockade of QUIS receptors, we have evaluated the effects of structural analogs of DNQX on ^{3}H-AMPA binding and on their ability to inhibit the hypermotility response to AMPH. DNQX, 2-hydroxy-3,5 dinitrotyrosine (DNT), and AFQX, an analog of DNQX, inhibited the binding of ^{3}H-AMPA with IC_{50} values of approximately 0.5 μM, 10μM, and 100 μM respectively. The doses of DNQX and DNT that inhibited AMPH-stimulated locomotion by 50% were 0.4 and 3.3 nmoles respectively, while AFQX at a dose of 5 nmoles did not exert an inhibitory effect. The order of potency for these compounds was similar for both inhibition of ^{3}H-AMPA binding and AMPH stimulated LMA. These studies suggest that the ability of DNQX to inhibit the hypermotility induced by AMPH is due to the blockade of QUIS receptors.

489.10

IN SITU HYBRIDIZATION OF OLIGONUCLEOTIDE PROBES TO mRNA ENCODING A KAINATE SENSITIVE GLUTAMATE RECEPTOR (GluR-K1). D.W. Bonhaus, D.A. Hosford, Z. Cao* and J.O. McNamara. V.A. and Duke Univ. Med. Ctrs. Durham NC 27705.

The recent isolation of cDNA clones encoding functional excitatory amino acid (EAA) receptors opens new avenues for investigating EAA receptor pharmacology. One key step in characterizing cloned receptors is to compare the anatomic distribution of the cloned molecule with pharmacologically and electrophsyiologically characterized endogenous receptors. Thus we used oligonucleotide probes to map the anatomic distribution of an mRNA which encodes a kainate activated, glutamate receptor. Two [^{32}P] labelled probes, complementary to non-overlapping regions of the GluR-K1 clone (Hollman et al.), were hybridized to 10 uM sections of rat brain. Northern blot analysis showed that the probes bound to identically sized bands of poly A^+ RNA. Similarly, the anatomic distribution of binding was identical for the two probes. The greatest density of binding was in the hippocampus; in hippocampus, binding was enriched in the dentate granule and CA3-1 pyramidal cell layers. The absence of a selective enrichment of binding over the granule or CA3 pyramidal cells argues against this mRNA encoding the high affinity [^{3}H]kainate binding site localized to area stratum lucidum. This suggests that the protein encoded by the GluR-K1 clone, while capable of generating kainate evoked currents, is not a part of the high affinity [^{3}H]kainate binding site. This in turn suggests that kainate evoked currents are mediated by a receptor other than that defined by high affinity [^{3}H]kainate binding. Which (if any) of the pharmacologically characterized EAA receptors includes the protein encoded by the GluR-K1 clone is unknown. However, the anatomic distribution of the mRNA together with the electrophysiologic properties of the expressed GluR-K1 clone raise the possibility that this protein may be part of a, quisqualate insensitive, AMPA receptor.

489.11

CHRONIC KAINATE TREATMENT DECREASES K^+-STIMULATED RELEASE OF ENDOGENOUS AMINO ACIDS FROM CULTURED CEREBELLAR NEURONS. M.L. Simmons and G.R. Dutton, Department of Pharmacology, University of Iowa, College of Medicine, Iowa City, IA 52242.

Cultured rat cerebellar neurons containing $\geq$90% granule cells, 5-7% inhibitory interneurons and 3-5% glial cells were used to identify the neuronal subclass of origin of released endogenous amino acids. Cultures were treated for 4 days, 5-8 days in vitro (DIV), with 50μM kainic acid (KA) which has previously been shown to selectively kill the GABAergic neuronal population (Drejer and Schousboe Neurochem. Res. 14:751-754, 1989). These conditions, under which a $\geq$80% loss of glutamic acid decarboxylase (GAD) immunoreactivity was seen, produced a complete loss of 50mM K^+-stimulated release of GABA, and a significant reduction in the release of aspartate, glutamate, taurine and adenosine at 9 DIV. These KA-induced effects were prevented by concurrent treatment with 50μM or 100μM, but not 10μM, 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX). Treatment with CNQX alone caused the release of all these substances above control levels.

Results indicate that glutamate and aspartate originate from granule neurons, GABA from inhibitory neurons, and adenosine and taurine possibly from both neuronal classes. KA treatment may decrease endogenous amino acid release from granule neurons by possibly depleting releasable pools or by downregulating KA receptors.

Supported by NS20632 and the Life and Health Insurance Medical Research Fund.

489.12

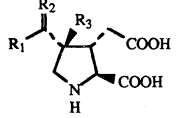
TOPA OXIDIZES IN SOLUTION TO FORM AN AMINO ACID WHICH IS A NON-NMDA AGONIST. D.S. Crawford, E. Aizenman, R.H. Loring, and P.A. Rosenberg. Dept. Physiol., U. of Pittsburgh Sch. Med., Pittsburgh, PA; Dept. Pharm., Northeastern U. and Depts. Med. and Neurol., Children's Hosp. and Harv. Med. Sch., Boston, MA 02115.

Application of solutions of 2,4,5 trihydroxyphenylalanine (topa) to rat cortical neurons or the chick eyecup preparation produces membrane responses which can be blocked by the non-NMDA antagonist CNQX (Soc Neurosci. Abstr., 15, 768, 1989). Topa is unstable in solution. Pharmacological activity depends upon the formation of an oxidation product. In order to identify the active compound, we have performed quantitative amino acid analysis with ninhydrin detection on solutions of dopa and topa, using a Beckman 7300 analyzer. Oxidized topa was prepared by dissolving topa in 50 mM sodium phosphate buffer at pH 6.8. Two peaks were found, one at the elution time for topa (20.7 minutes), containing 18% of the starting material, and a new peak at 10.3 minutes, containing 51% of the starting material. Incubation of the sample prior to analysis led to diminuition of this peak without appearance of new peaks. These data suggest that topa in solution oxidizes to a more stable amino acid, which is likely to be topa quinone; this derivative subsequently degrades to a substance which is not an amino acid. Of note, topa has recently been demonstrated in bovine plasma amine oxidase.

489.13

LY207328 AND LY207193: TWO POTENT KAINIC ACID AGONISTS. L. A. McQuaid, R. N. Booher*, D. D. Schoepp, M. M. Foreman and D. Lodge. Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Indiana 46285 and Royal Veterinary College, London, NW10TU, UK.

Kainic acid (R_1=CH_3, R_2=CH_2, R_3=H), isolated from *Digenea simplex*, is a powerful excitatory amino acid (EAA) agonist and neurotoxin. We wish to report two new potent kainic acid (KA) agonists, LY207328 (R_1=Ph, R_2=CH_2, R_3=H) and LY207193 (R_1=CH_3, R_2=NOH, R_3=H). LY207328 and LY207193 displace ^{3}H-KA with IC_{50}s of 4.0±0.1 nM and 36.5±0.8 nM, respectively. In addition, LY207192 (R_1=CH_3, R_2=CH_2, R_3=OH) demonstrated a more modest affinity for the ^{3}H-KA binding site with an IC_{50} of 526 nM. Both LY207328 and LY207193 were found to stimulate the release of ^{3}H-norepinephrine from rat hippocampal slices as well as the release of ^{3}H-choline from rat retinal tissue. LY207192 produced only a minimal response in the release assays. In electrophysiological studies on rat cortical wedges, LY207328 and LY207193 were potent depolarising agonists. These responses were sensitive to CNQX but not to CGS19755, suggesting an action via non-NMDA receptors as might be predicted from their structural similarity to KA.



490.1

NMDA-EVOKED OUTWARD CURRENTS IN CULTURED NEOCORTICAL NEURONS USING NYSTATIN-PERFORATED PATCH RECORDINGS. J.J. Hablitz and D.K. Mistry*. Neurobiology Research Center, University of Alabama at Birmingham, Birmingham, AL 35294.

The nystatin-perforated patch technique (Horn & Marty J. Gen. Physiol. V.92:145) was used to examine whole-cell responses to NMDA under conditions where loss of cytoplasmic factors was minimized and endogenous calcium buffering mechanisms were operational. The pipet contained (mM) 140 KC1 7 $MgC1_2$ 10 mM HEPES and 100 µg/m1 nystatin. At -60 mV, responses to 100 µM NMDA were biphasic, consisting of an initial inward current which decayed to a final steady-state value. No run down of either component was seen. Steady-state/peak ratios were similar in nystatin and conventional recordings suggesting similar rates of desensitization. When holding potentials from -40 to 0 mV were used, the initial inward current was followed by a slowly developing outward current. Near 10 mV, NMDA evoked an initial small decaying outward current followed by a large sustained outward current. At more positive holding potentials, NMDA evoked an initial outward current which decayed to a steady-state value. If cesium was substituted for potassium in the pipet, NMDA-induced outward currents were not observed.

Thus, under conditions where endogenous calcium buffering mechanisms are operational, NMDA evokes an outward current in cultured neocortical neurons. This is attributed to activation of a Ca-dependent K current via Ca entry through NMDA channels. (Supported by NS18145)

490.2

WHOLE CELL PATCH-CLAMP RECORDING OF ENDOGENOUS SYNAPTIC CURRENTS IN MAMMALIAN MOTONEURONS IN INTACT BRAINSTEM-SPINAL CORD. Guosong Liu & Jack L. Feldman. System Neurobiology Laboratory, Dept of Kinesiology, UCLA, Los Angeles, CA 90024-1568.

We previously reported that an excitatory amino acid (EAA)-like substance is the neurotransmitter involved in the bulbospinal transmission of inspiratory drive to phrenic motoneurons (Liu & Feldman, Soc. Neuro. Abs. 15:643, '89; Liu et al. J. Neurophysiol. In Press). To further characterize this endogenous excitatory synaptic transmission, whole cell patch-clamp techniques were employed in a brainstem-spinal cord preparation *in vitro (ibid.)*. We used standard intracellular recordings to locate the phrenic motoneuron pool, then lowered a patch electrode (~3-5 MΩ) into the pool (~180-260 µm from surface). The tip of the patch electrode was kept clean by continuous positive pressure. Gigaohm seals (~1-5 GΩ), and whole cell patch clamp, were obtained by suction. The measured input resistances of these motoneurons were 100-500 MΩ, compared to values of ~20-50 MΩ from standard intracellular recordings. The electrode was judged to be attached to the soma if large whole cell capacitance, and appropriate shape and voltage-dependence of the fast Na current, were measured Two types of synaptic currents were found. One occurs during inspiration and is mediated by an EAA-like transmitter. The associated EPSCs had very fast rise-time (< 0.2 ms) and decay-time (~3-5 ms) constants, reversal potentials near 0 mV, and were blocked by the non-NMDA receptor antagonist CNQX. The change of shape of EPSCs at different holding potentials suggest they are voltage-dependent. Another synaptic current was present during expiration. This current had slower rise-time and longer decay-time (~40 ms) constants and reversed near -40 mV. We interpret the later current as an inhibitory synaptic current carried by $GABA_A$ channels.

In summary, whole cell patch clamp technique is feasible in neurons receiving **endogenous** synaptic drive. This should permit the study of synaptic transmission under more physiological conditions than in preparations that require stimulated release of neurotransmitter. Supported by NIH Grant NS 24742.

490.3

QUINOLINIC ACID EFFECTS ON HIPPOCAMPAL PYRAMIDAL NEURONS. J.E.Monckton*, N.T.Carnevale and J.J.Halperin. Departments of Neurology and Neurobiology, SUNY, Stony Brook, NY 11794.

We examined effects of quinolinic acid (QA) on pyramidal neurons in the in vitro rat hippocampal slice. At concentrations >= 100µM, this NMDA agonist was reported to decrease the amplitude of antidromically evoked action potentials (APs) recorded extracellularly in the CA1 region (bath application [Stone]), and to depolarize cultured hippocampal neurons (pressure ejection from micropipette [Tsuzuki et al.]).

Our extracellular recordings from CA1 found that bath application of 100-200µM QA depressed or abolished the EPSP and population spike evoked by stimulating the Schaffer collaterals ([Mg++]o = 2mM). Intracellular recordings disclosed that QA reversibly depolarized CA1 pyramidal neurons to a variable degree, and attenuated or eliminated EPSPs. Injecting depolarizing currents triggered slow, regenerative depolarizing waves. In some cells, QA eliminated APs evoked synaptically or by current injection. These effects were reversibly blocked by APV, and resolved after washing with Ringer.

This work was supported in part by the Department of Veterans Affairs (NTC) and by a grant from New York State for Lyme Disease research (JJH).

490.4

UNILATERAL RHYTHMIC VIBRISSAE MOVEMENTS INDUCED BY KAINIC ACID INJECTED IN DEEP STRATA OF SUPERIOR COLLICULUS. B. S. Grunwerg*, J. Krol*, M. M. Corton* and G. M. Krauthamer. Dept. of Neuroscience and Cell Biology, UMDNJ, Robert Wood Johnson Med. Sch., Piscataway, N.J. 08854.

During the course of an unrelated study we made the unexpected observation that the injection of small amounts of kainic acid in the deeper strata of the anterior third of the superior colliculus induced an acute and transient phase of rhythmic whisker movements. Since the vibrissae are extensively represented in this part of the superior colliculus and its neurons entertain both bilaterally descending as well as asending projections we believe that this observation may be of some interest.

Nine Long--Evans rats were anesthetized with 350 mg/kg chloral hydrate. Two to four unilateral injections of 0.5 ul kainic acid (3.52 nM) were made at 2-5 min intervals into the intermediate and deep strata. Within 1-3 min of the last injection, a 2-5 Hz rhythmic whisker movement developed lasting about 5-15 min. The movement was always confined to the vibrissae ipsilateral to the injection site; in some animals it included the ipsilateral nostril. No other movements could be observed during this time. Neither the EEG nor bipolar recordings from the hippocampus indicated typical seizure activity nor could we detect any hippocampal neuronal damage in rats sacrificed within one hour of the excitotoxin injections.

At this time we cannot entirely rule out the possibility of an abortive limbic seizure due to the susceptibility of the hippocampus to kainic acid action "at a distance". On the other hand, the possibility should be considered that the induced rhythm reflects the transient activation of glutamatergic kainate receptors of tectal neurons. (Supported by NS20626).

490.5

NMDA RECEPTORS MEDIATE A COMPONENT OF GRANULE CELL RESPONSES TO LOW FREQUENCY STIMULATION OF PERFORANT PATH AFFERENTS *IN VIVO*. T.A. Blanpied, G. Barrionuevo, and T.W. Berger. *Depts. of Behavioral Neurosci. and Psychiatry, U. of Pittsburgh, Pgh., PA, 15260.*

Previous studies using *in vivo* preparations have reported that extracellular EPSPs recorded in the hippocampal dentate gyrus in response to low frequency (<0.2Hz) stimulation of the medial perforant path are not reduced by the selective NMDA receptor antagonist D-APV. However, intracellular studies of the dentate *in vitro* have found that CNQX, an antagonist selective for kainate/quisqualate receptors eliminates all but a small D-APV sensitive portion of the granule cell EPSP. We examined population EPSPs recorded from the molecular layer of the rabbit dentate gyrus *in vivo*, while administering CNQX, D-APV, or saline by microinfusion through a multibarrelled pipette positioned 100-500µm from the recording site.

CNQX (25-50µM) reduced the evoked EPSP by 70-95% (n=12) without affecting the afferent fiber volley; however, in all cases tested (n=8), subsequent addition of D-APV (50-100µM) substantially reduced the remaining response. For greater resolution of the component sensitive to D-APV, we used digital subtraction of responses evoked during the application of CNQX and D-APV from those evoked in the presence of CNQX alone. The residuals clearly revealed that D-APV eliminated a negative-going potential with a peak latency 25-200% longer than the control peak ($\bar{x}$=10.0ms vs 5.1ms control) and a peak amplitude of up to 20% ($\bar{x}$=11%) of the control amplitude. In separate experiments, D-APV was applied alone and evoked responses were subtracted from those recorded in the presence of saline. These remainders had a time course very similar to the CNQX-APV residuals. In 4 experiments in which responses were recorded simultaneously from the cell body and molecular layers, the D-APV sensitive components observed in the two laminae were of opposite polarity. Parametric manipulations (n=8) indicated that the amplitude of this component is strongly frequency dependent, and is greatly enhanced (200% of single impulse amplitude) in response to 2-5 impulses delivered with a 2ms interstimulus interval.

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490.6

REDUCTION OF ACTIVITY OF THE GLYCINE SITE AT THE NMDA RECEPTOR COMPLEX INTERFERES WITH THE INDUCTION OF LTP *IN VIVO*. E. Thiels, D. J. Weisz and T. W. Berger. *Departments of Behavioral Neuroscience and Psychiatry, University of Pittsburgh, Pittsburgh, PA 15260.*

Glycine has been shown to markedly potentiate synaptic events mediated by the NMDA receptor complex in *in vitro* preparations. Conversely, antagonist activity at the glycine site has been demonstrated to block NMDA receptor-mediated responses *in vitro*, which suggests that activation of the glycine site is necessary for NMDA receptor-mediated events. We examined whether or not the selective glycine site antagonist, 7-chlorokynurenic acid (7-Cl-Ky), prevents the induction of LTP at the commissural-CA1 synapse of the rat hippocampus *in vivo*, and furthermore, whether or not antagonist effects could be overcome by concurrent application of the glycine site agonist, D-serine.

Microinjection 400-500µm from the recording site of 7-Cl-Ky at doses ≤ 200µM (n=4) did not notably alter the population spike (PS) of CA1 pyramidal cells evoked by low frequency (0.1 Hz) stimulation of commissural fibers, and prevented the induction of LTP following high frequency stimulation (100Hz for 1s) in only one case. PS amplitude at threshold level increased from M=0.3 to 2.4mV, compared to an increase from M=0.2 to 5.1mV during injection of vehicle solution (n=4) or a change from M=0.3 to 0.1mV during injection of the selective NMDA receptor antagonist, D-APV (100µM; n=4). Application of 7-Cl-Ky at a dose of 400µM (n=4) slightly increased PS threshold and attenuated the induction of LTP in three cases; PS amplitude increased from M=0.2 to 1.1mV. Injection of 400µM 7-Cl-Ky combined with 1mM D-serine (n=3) did not alleviate the effect of 7-Cl-Ky on PS threshold, but reinstated the induction of LTP; PS amplitude increased from M=0.2 to 4.1mV. We conclude that activity at the glycine site can modulate NMDA receptor activation in the intact brain. (Supported by NIMH: MH45156 and MH00343, and NSF: BNS8945137).

490.7

ROLE OF EXCITATORY AMINO ACIDS IN THE NEURAL CIRCUIT MEDIATING THE ACOUSTIC STARTLE REFLEX. M.J.D. Miserendino, N.M. Boulis, R.F. Spiera, and M. Davis. Dept. of Psychiatry, Yale Univ. Sch. of Med., 34 Park St., New Haven, CT. 06508

Acoustic startle is a short latency reflex elicited by a brief auditory stimulus. The neural circuit mediating the acoustic startle reflex is thought to consist of: the ventral cochlear nucleus (VCN); the paralemniscal zone, an area just medial to the ventral lateral lemniscus (VLL); the nucleus reticularis pontis caudalis (RPC); and motor neurons of the lumbar spinal cord. Infusion of selective competitive excitatory amino acid antagonists into these sites causes significant and dose-dependent decreases in the magnitude of the startle response.

Specifically, local infusion into the VCN showed that while the non-NMDA antagonist CNQX (6-cyano-7-nitroquinoxaline-2,3-dione) attenuated the startle reflex, the NMDA-specific antagonists AP5 (DL-2-amino-5-phosphonopentanoic acid) and CPP (3-((+)-2-caboxypiperazin-4-yl)-propyl-1-phosphonic acid) were without effect. In the VLL or RPC, NMDA and non-NMDA antagonists were equipotent in blocking startle; however, RPC showed a much greater sensitivity to these drugs than other startle circuit sites. Intrathecal infusion of AP5 and CNQX into the lumbar cord region again showed both these compounds to be about equipotent in attenuating whole-body startle. A finer analysis of acoustically elicited startle measured electromyographically from the quadriceps femoris complex in the hindlimbs showed that intrathecal CNQX preferentially blocked an early, short latency (~8 msec) EMG component of startle, while AP5 preferentially blocked a late (~15 msec) component.

These findings indicate that excitatory amino acids may mediate or modulate the acoustic startle reflex at each nucleus within its neural pathway, and suggest that the temporal and relative contribution of NMDA and non-NMDA receptors, and their sensitivity, may vary as a function of site.

490.8

ELECTROPHYSIOLOGICAL EVIDENCE THAT A TAURINE-LIKE AMINO ACID IS THE NEUROTRANSMITTER AT A FAST EXCITATORY SYNAPSE. Peter A.V. Anderson and H.G. Trapido-Rosenthal. Whitney Laboratory and Depts of Physiology and Neuroscience, Univ. of Florida, St. Augustine, FL 32086.

Neurons in the motor nerve net of the jellyfish *Cyanea capillata* are connected by fast relay synapses. The average delay between the peak of the presynaptic action potential and the onset of the EPSP is 1 msec suggesting that the neurotransmitter at these synapses cannot be peptidergic, as appears to be the case at many cnidarian synapses. Previous work involving applications of 25 putative transmitter substances to exposed synapses failed to evoke any activity consistent with the normal EPSPs at these synapses. However, applications of taurine and ß-alanine, but not alanine, and some, but not all analogs, evoked reproducible depolarizations of the cells. These were associated with a conductance increase. The reversal potential of the response averaged +6.8 mV, close to the average reversal potential of EPSPs at these synapses (+4 mV). This physiological evidence for a role of a taurine-like transmitter is supported by finding that neurons from this animal contain large amounts of taurine, ß-alanine, alanine and GABA. If release studies, currently underway, confirm the role of a taurine-like neurotransmitter at these synapses, this will constitute the first convincing demonstration of their role as neurotransmitters.
Supported by NSF grant BNS-8805885.

490.9

DESCENDING AND SEGMENTAL GLUTAMATERGIC INPUTS MEDIATE TWO DISTINCT EPSPs IN RAT SYMPATHETIC PREGANGLIONIC NEURONS. E. Shen, N. Mo* and N. J. Dun. Dept. of Pharmacol., Loyola Univ. Med. Ctr., Maywood, IL 60153

Intracellular recordings were made from antidromically identified sympathetic preganglionic neurons (SPNs) in thin (500 µm) transverse neonate (12-22 day) rat thoracolumbar spinal cord slices. Electrical stimulation of dorsal roots elicited in SPNs a long latency (1-5 ms), relatively slow rising and falling EPSP, referred to as a DR-EPSP. Hyperpolarization and depolarization from the resting potential of about -60 mV reduced and increased the DR-EPSPs. The response was potentiated in Mg-free solution, often giving rise to a burst of spike discharges. DR-EPSPs in the presence or absence of Mg ions were suppressed by the NMDA receptor antagonists APV (1-10 µM) and ketamine (10-20 µM) but resistant to the KA/QA receptor antagonists DNQX and CNQX (1-10 µM). Stimulation of lateral funiculus evoked a short latency (< 1 ms), relatively fast rising and falling EPSP, the LF-EPSP. The responses were increased by hyperpolarization and reduced by depolarization. Mg-free solution had no appreciable effects on LF-EPSPs. Contrary to the DR-EPSP, the LF-EPSP was antagonized by DNQX and CNQX but not by APV and ketamine. The results indicate that SPNs receive two glutamatergic inputs: a polysynaptic connection from dorsal roots and a monosynaptic input from supraspinal neurons via lateral funiculus. Activation of these two inputs elicits two distinct EPSPs mediated by NMDA and non-NMDA receptors, respectively. (Supported by NS18710).

490.10

ELECTRICAL ACTIVITY IN THE SUPRACHIASMATIC NUCLEUS, EVOKED BY OPTIC TRACT STIMULATION, CAN BE RECORDED USING VOLTAGE-SENSITIVE DYES. H. Komuro*, A.L. Obaid, and B.M. Salzberg, Dept. of Physiology, Univ. of Pennsylvania School of Medicine, Phila., PA 19104-6085.

In an effort to understand the functional organization of the suprachiasmatic nucleus (SCN), we have used a system for Multiple Site Optical Recording of Transmembrane Voltage (MSORTV) and the potentiometric pyrazo-oxonol dye RH 155 to monitor electrical events that follow stimulation of the retino-hypothalamic pathway. Coronal slices, 300 um thick, were cut at the level of the optic chiasm of adult mice, stained for 30 minutes in a 0.2 mg/ml solution of the dye, and imaged onto a 124 element photodiode array using a 10X, 0.4 n.a. objective. Brief stimuli (100-200 us) were delivered to the optic tract by means of a bipolar electrode. Each photodetector monitored changes in transmitted light intensity which were proportional to the potential changes of the plasma membranes in its 100 um square receptive field. These extrinsic optical signals derived mainly from retinal ganglion cell axons and neurons of the SCN, and exhibited waveforms having at least two components whose relative sizes depended upon location within the slice as well as the origin of the slice on the rostral-caudal axis. The first component was very fast, TTX-sensitive, and appeared to be dominated by the axonal spike. The slower portion of the optical signal had a wider distribution in more rostral slices. It was sensitive to high (11 mM) $[Mg^{2+}]_o$ and to blockers of glutaminergic transmission such as kynurenic acid (1 mM), and a spatial organization of these effects could be discerned. 6-Cyano-7-nitroquinoxaline-2,3-dione (CNQX) eliminated the slow component at a concentration of 20 uM. These findings suggest that the second component of the absorption change reflects postsynaptic voltage changes in SCN neurons.

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490.11

HOW NMDA AND NON-NMDA RECEPTORS CONTRIBUTE TO RESPONSES IN VISUAL CORTEX. N.W. Daw and K. Fox. Dept. Cell Biol., Washington Univ. Med. Sch., St. Louis, MO 63110

We have observed the effect of glutamate agonists and antagonists on contrast response curves of cells in cat visual cortex. NMDA increases the slope of the curve without changing the levels of contrast that give threshold and saturation, while APV reduces the slope and quisqualate shifts the curve upwards.

We have developed a model that accounts for these results using parameters for the cell biology of glutamate receptors from the hippocampus. The NMDA channel conductance has a Boltzmann voltage dependancy with exponent -0.07V, while glutamate binds at non-NMDA and NMDA receptors with Hill coefficients of 1 and 2 respectively. Release of glutamate from terminals in the cortex is assumed to be related to contrast by a hyperbolic tangent formula, C/C+K, where C is contrast and K is a constant.

Several conclusions can be drawn. NMDA receptors contribute to the visual response multiplicatively, while non-NMDA receptors contribute additively. The NMDA receptor is active at low as well as high contrasts, accounting for a constant percentage of the response at all contrasts. It does not act as a switch that turns on Calcium inflow at some particular level of input. Consequently NMDA receptors contribute to the visual response in both adult and developing visual cortex in a graded fashion.

490.12

CHARACTERIZATION OF GLUTAMATE RECEPTORS AND THEIR MODULATION BY VASOPRESSIN IN RAT HYPOTHALAMIC MAGNOCELLULAR NEUROSECRETORY CELLS (MNCS). B. Hu and C.W. Bourque *Centre for Research in Neuroscience, MGH, McGill University, Montreal, Québec, Canada H3G 1A4*

Glutamate receptor antagonists have been shown to block evoked excitatory synaptic transmission in the supraoptic nucleus (Gribkoff et al, J. Neurophysiol. 1990). Experiments on ventral septal neurons have previously shown that glutamate evoked firing can be modulated by vasopressin (VP) (Disturnal et al, CJPP 1987). Since 50% of supraoptic MNCs release VP, we have characterized the glutamate receptor subtypes on supraoptic neurons and examined the effects of VP on their activation by selective agonists. Forty eight supraoptic MNCs were recorded using current- or single electrode voltage clamp techniques. Bath applications of NMDA, quisqualate, kainate or glutamate consistently induced a dose-dependent, TTX-insensitive inward current or membrane depolarization (n=11) at resting potential. The I-V relationship of the NMDA-induced current revealed a Mg^{++}-dependent zone of negative resistance between -100 to -45mV (n=9). In contrast, currents evoked by quisqualate and kainate displayed linear I-V relationships. NMDA induced responses were reversibly antagonized by APV (10 to 40 µM; n=5), while quisqualate responses were selectively blocked by CNQX (10 to 20 µM; n=4), suggesting that both NMDA and non-NMDA receptors are present on MNCs. Bath application of vasopressin (10^{-8} to 10^{-6} M) reversibly attenuated NMDA induced responses (50% to 100%; n=7) in a concentration-dependent manner. In contrast, quisqualate induced responses in the same cells were unaffected or enhanced by VP (n=5). The modulatory effects of VP may be relevant to the physiological control of supraoptic neurons as well as their peculiar resistance to glutamate toxicity. Supported by MRC, FCAR and FRSQ

490.13

NOVEL ACTIVATION OF NMDA RECEPTORS POTENTIATES SENSORY RESPONSES OF BRAIN NORADRENERGIC NEURONS. R. Shiekhattar and G. Aston-Jones. Div. Behav. Neurobiol., Dept. Mental Health Sci., Hahnemann University, Philadelphia, PA 19102.

Noradrenegic locus coeruleus (LC) neurons receive prominent excitatory amino acid (EAA) and GABA inputs from their two major afferents in the rostral medulla (Ennis and Aston-Jones, J. Neurosci., 8: 3644 ; 9 : 2973 1988;1989), and the characteristic activation of these cells by sciatic nerve stimulation is mediated by an EAA in LC. Using this system, we examined possible GABAergic modulation of synaptic EAA-mediated transmission *in vivo*. Extracellular recording was combined with local microinfusion of drugs into LC. Direct application of the GABA antagonist bicuculline methiodide (BIC; 50 or 500 μM) onto LC neurons enhanced their sensory responsiveness by 234% ($p<.004$, $n=7$). This increased responsiveness was due to the long-lasting expression of a new, N-methyl-D-aspartate (NMDA) receptor-mediated component of the synaptic response, as it was completely blocked by infusions of the specific NMDA antagonist, 2-amino-5-phosphonovalerate (AP5). This action of BIC was neither mimicked by other GABA-A (picrotoxin or penicillin) or GABA-B (2-hydroxy-saclofen) antagonists, nor by agents that directly depolarize LC neurons (vasoactive intestinal polypeptide or carbachol). This BIC-potentiated response component was eliminated by direct application of the neurotransmitter GABA.

These results indicate that BIC, acting at a novel site, unmasks NMDA receptors which can be activated by sensory stimuli. This new site may be a point of convergence whereby interactions between two major neurotransmitter systems potently modulate signal transmission in the brain. Support: PHS grants NS24698 & DA06214.

490.14

THE BLOCKADE OF POTASSIUM CONDUCTANCES BY EXCITATORY AMINO ACIDS IS MEDIATED BY A SUBTYPE OF QUISQUALATE RECEPTOR

S. Charpak*, T. Knöpfel, S. Thompson and B.H. Gähwiler. Brain Research Institute, University of Zürich, CH-8029 Zürich (Switzerland)

Recently, we reported that cysteine could block the slow spike afterhyperpolarization (AHP) of hippocampal CA3 pyramidal neurones (Charpak et al. Soc. Neurosci. abstr. 374.13. 1989). In the present work, we characterized the endogenous agonists, receptor type and membrane currents involved in this effect.

Organotypic cultures of rat hippocampus were prepared as described previously. After 2-5 weeks in vitro, the cultures were superfused with a balanced salt solution containing 1 uM TTX and single-electrode voltage-clamp techniques were used to record currents from CA3 pyramidal cells.

Glutamate, aspartate, cysteine and homocysteate (0.5-1 mM) blocked a calcium-dependent potassium current underlying the AHP and the time- and voltage-dependent current I_M, despite pharmacological blockade (2 mM kynurenate) of ionotropic excitatory amino acid receptors. These effects could be reproduced by low concentrations (0.02-1 uM) of quisqualate and by agonists of the quisqualate metabotropic receptor such as Trans-1-amino-cyclopentyl-1,3-dicarboxylate. Combined intracellular and fura-2 recordings indicated that the quisqualate effect was not mediated through a change in cytosolic calcium, thus suggesting a direct blockade of potassium conductances.

490.15

ISOFLURANE DEPRESSION OF Ca-COMPONENT INVOLVED IN NMDA AND GLUTAMATE ACTIONS ON CORTICAL NEURONS.

H. El-Beheiry, E. Puil and K. G. Baimbridge. Depts. of Anaesthesia, Pharmacology & Therapeutics, and Physiology, Univ. of British Columbia, Vancouver, B. C., V6T 1W5, Canada.

A decrease in synaptic excitation has been suggested as a mechanism of action of general anaesthetics. The effects of isoflurane were studied using intracellular recording techniques and microspectrofluorimetric measurements of intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) with a Fura-2 probe in neocortical and cultured hippocampal neurons. In the presence of external Mg^{2+} (2 mM), isoflurane depressed, in a dose-dependent manner, the depolarizations and associated conductance changes evoked by iontophoretic applications of NMDA and glutamate (Glu). Anaesthetic administrations in the lower dose range (0.5-1.5MAC) had no effects on the passive membrane properties of the neocortical neurons. In higher doses, isoflurane induced slight hyperpolarization (3-5 mV) as well as increased input conductance (≈20%). In hippocampal neurons, isoflurane attenuated, in a dose-dependent manner, the increases in $[Ca^{2+}]_i$ evoked by bolus application of Glu or NMDA under conditions that favoured activation of the NMDA- and quisqualate-receptor subtypes. Verapamil perfusion reduced the presumed voltage-dependent increases of $[Ca^{2+}]_i$ induced by Glu application. The effects of isoflurane were additive with those of verapamil. It is suggested that isoflurane suppresses the depolarizations induced by NMDA and Glu by actions on receptor- and voltage-dependent Ca2+ influx or on processes for intracellular Ca2+ mobilization activated by Glu-receptor interactions.

490.16

ALLOSTERIC MODULATORS OF THE NMDA RECEPTOR AFFECT SYNAPTIC FUNCTION IN RAT HIPPOCAMPAL SLICES. S. Vicini, C.T. Livsey and G. Mereu. FGIN, Georgetown University, Washington, D.C. 20007.

Excitatory synaptic transmission in the dentate gyrus granular neurons and CA1 and CA3 pyramidal neurons of the rat hippocampus in a thin slice preparation (Edwards et al. Pfluger Arch. 414: 600, 1989) was studied by mean of the whole-cell tight-seal technique. We observed miniature spontaneous excitatory synaptic currents (mepcs) in most neurons investigated. Low frequency stimulation of the afferent input to these areas produced evoked excitatory synaptic currents (epsc). The synaptic currents of both mepcs and epcs presented in addition to a main fast decay a slower component (20-300 msec duration) relevant at holding potentials more depolarized than -55 mV, due to the relief of the Mg^{2+} blockade of NMDA-activated receptor-channels. Negative allosteric modulators for the glycine site located on the NMDA receptor domains such as 7-Cl-kynurenic acid abolished the slow component of the epsc. Tetanization of the excitatory input to dentate gyrus granular cells and CA1 pyramidal neurons produced in most cases long-lasting facilitation of the epscs and in some cases also of the mepscs if combined with depolarization of the postsynaptic neurons in the presence of picrotoxin. 7-Cl-kynurenic acid prevented the induction of this facilitation. These results demonstrate a role for the glycine regulatory site in the excitatory synaptic transmission in the hippocampus.

This work is supported by an NIH grant # PO1 NS-78130.

490.17

GLYCINE MODULATES EXCITATORY AMINO ACID-INDUCED EXCITATION OF RAT CEREBELLAR PURKINJE CELLS IN VIVO.

J.G. Netzeband, J.C. Strahlendorf and H.K. Strahlendorf. Departments of Physiology and Neurology, Texas Tech University Health Sciences Center, School of Medicine, Lubbock, TX 79430.

The action of glycine on excitatory amino acid-induced excitation of cerebellar Purkinje cells was investigated in urethane-anesthetized, adult, male rats using extracellular recording techniques and iontophoretic drug application. Glycine, alone, had little or no effect on the spontaneous firing rate of Purkinje cells, but it dose-dependently attenuated kainate-and quisqualate-induced excitations. Glycine's action on NMDA-induced excitation, however, was more complex and dependent on the initial firing rate of the cell. Slower firing cells responded with a dose-dependent attenuation, similar to that observed above. Faster firing cells, however, showed potentiation of NMDA-mediated excitation in the presence of a low dose of glycine (5 nA). In these cells, higher doses of glycine (10-40 nA) were less effective at potentiating NMDA, and in several cells, the effect was reversed to one of attenuation. These results suggest that glycine elicits a non-selective inhibitory modulation of excitatory inputs onto Purkinje cells, and that it also selectively potentiates responses to NMDA. This selective action on NMDA is in agreement with data from other areas of the nervous system which show that the NMDA-receptor has an allosteric glycine binding site and lends further support to the hypothesis that functional NMDA receptors exist on adult cerebellar Purkinje cells. Supported by NS19296 and the Tx. Adv. Res. Prog, Grant 010674-020.

490.18

INCREASED SENSITIVITY TO NMDA FOLLOWING CHRONIC LEAD EXPOSURE.

T.L. Petit, J.C. LeBoutillier*, and W.J. Brooks. Dept. of Psychology, Univ. of Toronto, Scarborough, Ont. Canada M1C 1A4

Exposure to lead (Pb) in children and developing animals inhibits neuronal development, adult plasticity, and learning and memory capacities. The NMDA receptor is critical in neural development, plasticity, and memory processes, and is known to be altered by divalent cations. The current research attempted to determine if there was an interaction between Pb and NMDA. Rat pups were exposed to high, moderate, and low levels of Pb during early development and injected with varying doses of NMDA at P15 or P25. Lead exposed rats were supersensitive to NMDA showing a more rapid onset and intense level of seizure activity that was relative to the degree of Pb exposure. Receptor binding autoradiography indicated an increase in NMDA receptors in the Pb exposed animals, particularly in the hippocampus. These effects are similar to those produced following chronic developmental administration of NMDA antagonists, and suggest that developmental Pb exposure chronically disrupts NMDA receptor function, resulting in a compensatory up-regulation of NMDA receptors.

490.19

SYNAPTOGENESIS AND ENHANCED SENSITIVITY TO NMDA FOLLOWING CHRONIC ADMINISTRATION OF PCP.
W.J. Brooks, T.L. Petit and J.C. LeBoutillier*
Dept. of Psychology, Univ. of Toronto, Scarborough, Ont, Canada, M1C 1A4.

Previous research in our laboratory has found that chronic administration of the NMDA antagonist phencyclidine (PCP) inhibits developmental synaptogenesis. The current research attempted to determine the effects of terminating chronic administration of PCP on subsequent sensitivity to NMDA and synaptogenesis. Five day old rat pups were administered daily subcutaneous injections of 10 mg/kg PCP for two weeks (the period of maximal neocortical synaptogenesis). The pups were sacrificed 1, 5, 15 and 25 days after the last injection of PCP and cortical sections processed for electron microscopy. Analysis of the molecular layer of occipital cortex revealed an initial (21) drop in total number of synapses followed by a period in which total synapses exceeded control values (P36). This period of synaptogenesis coincided with an enhanced sensitivity to NMDA induced seizure activity. These results provide further evidence of a relationship between NMDA sensitivity and the ongoing rate of synaptogenesis.

490.20

PHORBOL ESTER ENHANCES EXCITATORY AMINO ACID-INDUCED DOPAMINE RELEASE. I. Chaudieu, H. Mount, & P. Boksa. Douglas Hospital Research Center, Depts. Psychiat. and Pharmacol. & Therap., McGill University, Verdun, PQ., Canada.

Several neurotransmitter systems mediate cellular events via induction of phosphatidyl inositol breakdown leading to activation of protein kinase C (PKC). In support of this, phorbol esters, known to activate PKC, have been shown to enhance processes such as neurotransmitter release and features of long term potentiation. In fetal rat cultured mesencephalic cells, we show that the phorbol, 12-O-tetradecanoyl phorbol-13-acetate (TPA), at 100 nM, enhanced [^{3}H]dopamine ([^{3}H]DA) release evoked by NMDA, kainate, quisqualate or K^+ to 160%, 127%, 173% and 167% of control values, respectively. 100 nM TPA alone had no effect on [^{3}H]DA release. [^{3}H]DA release evoked by NMDA in the presence of TPA was completely inhibited by the non-competitive NMDA antagonist, MK-801, indicating that TPA enhancement of the NMDA response is mediated via activation of NMDA receptors. TPA enhanced NMDA-stimulated [^{3}H]DA release both in the presence and absence of 1.2 mM Mg^{2+}, indicating that TPA does not enhance the NMDA response by depolarizing DA neurons leading to reversal of a voltage-dependent Mg^{2+} blockade of the NMDA response. TPA also does not enhance the NMDA response in this preparation via release of glycine leading to glycine potentiation of the NMDA response, since in the presence of Mg^{2+}, glycine (1 or 10 uM) had no effect while 100 uM glycine inhibited NMDA-induced [^{3}H]DA release (Mount et al., this meeting). These results suggest that PKC activation may modulate excitatory amino acid-induced DA release from neurons originating in the ventral mesencephalon. *Supported by the FRSQ.*

490.21

PRESYNAPTIC GLUTAMATE RECEPTORS MODULATE NEUROTRANSMITTER RELEASE FROM SYNAPTOSOMES.
James K.T. Wang and Vijay Thukral*. Tufts University School of Medicine, Boston, MA 02111.

The existence of presynaptic glutamate receptors, and the question of which of the glutamate receptor subtypes regulates transmitter release, were addressed in this study. We labeled synaptosomes from adult rat olfactory bulbs with [^{3}H]norepinephrine, [^{3}H]dopamine, or [^{14}C]GABA, and assayed transmitter release in a superfusion apparatus. Glutamate (10 μM) increased the basal release of the two catecholamines by 50% within 1-2 min, and did not have any sigificant effect on the K^+-depolarization evoked release. The enhanced basal release was dependent on extracellular Ca^{2+}, and was blocked by the non-NMDA subtype selective antagonist CNQX (10 μM) but not by the NMDA subtype selective antagonist APV (10 μM). Quisqualate (10 μM) and AMPA (100 μM) had similar effects as glutamate, but kainate and NMDA did not. Glutamate also increased both the basal and the K^+-depolarization evoked efflux of [^{14}C]GABA. However, these effects were not blocked by either CNQX or APV, were not mimicked by AMPA, kainate or NMDA, and were not dependent on extracellular Ca^{2+}. These data suggest that in olfactory bulb synaptosomes there are presynaptic glutamate receptors, possibly of the non-NMDA quisqualate/AMPA subtype, that modulate catecholamine release. In contrast, the effects of glutamate on GABA efflux may not involve the Ca^{2+}-dependent, releasable pool of the transmitter.
(Supported by the Pew Charitable Trust)

EXCITATORY AMINO ACIDS: ANATOMY AND PHYSIOLOGY II

491.1

CEREBRAL SYNTHESIS AND RELEASE OF KYNURENIC ACID
K.J. Swartz, M.J.During, A.Freese and M.F.Beal *Program in Neuroscience, Harvard Medical School, and Mass.General Hospital, Boston MA 02115.*

Kynurenic acid is an endogenous antagonist of excitatory amino acid (EAA) receptors and may therefore influence important physiologic and pathologic processes. The release of intracerebrally synthesized kynurenic acid into the extracellular fluid (ECF), where it may act at EAA receptors, has not been established *in vivo*. Furthermore, kynurenic acid synthesis from physiologically supplied precursors has not been demonstrate *in vivo* or *in vitro*. We studied the synthesis and release of kynurenic acid in rat striatum, using intracerebral microdialysis coupled with HPLC and fluorescence detection. The basal ECF concentration of kynurenic acid in the rat striatum was 17.1 ± 1.1 nM. Peripheral administration of the immediate precursor of kynurenic acid, L-kynurenine, resulted in marked dose dependent increases in striatal ECF concentrations of kynurenic acid, peaking at 2.5 hrs. The highest dose of L-kynurenine (100 mg kg^{-1}), administered peripherally, resulted in a 108 fold increase in plasma kynurenic acid levels and a 37 fold increase in cerebral ECF levels. Peripheral administration of kynurenic acid, at a dose that caused plasma levels to increase 430 fold, resulted in only 4 fold increases in striatal ECF concentrations. The precursor responsiveness of striatal ECF kynurenic acid to peripherally infused L-kynurenine was blocked by the central application (via the dialysis probe) of 1mM aminooxyacetic acid, an inhibitor of the immediate synthetic enzyme for kynurenic acid, kynurenine aminotransferase. Administration of L-tryptophan, was less effective than L-kynurenine in increasing ECF kynurenic acid concentrations, and did so at a considerably later time interval (6 hrs.). Infusion of L-kynurenine (100 µM), but not L-tryptophan (100 µM), through the dialysis probe, dramatically increased striatal ECF concentrations of kynurenic acid. The conclusions drawn from the present study are that: i) kynurenic acid is present in ECF within the central nervous system (CNS), ii) the CNS can synthesize kynurenic acid and release it into the ECF, iii) the majority of CNS kynurenic acid synthesis results from the transport of L-kynurenine across the blood brain barrier and iv) ECF concentrations of kynurenic acid can be dramatically increased by pharmacological manipulation of precursor levels.

491.2

APNEA PRODUCED BY MICROINJECTION OF KYNURENIC ACID INTO THE CAUDAL-SUBRETROFACIAL AREA OF THE VENTROLATERAL MEDULLA IN THE CAT IS DUE TO BLOCKADE OF BOTH NMDA AND NON-NMDA EXCITATORY AMINO ACID RECEPTORS.
R.A. Gillis*, P.J. Hornby and T.P. Abrahams, Dept. of Pharmacology, Georgetown University, Washington, D.C. 20007.

We recently reported that bilateral microinjection of kynurenic acid (KYN; 12.5nmol in 50nls) into the caudal-subretrofacial area produces apnea in chloralose anesthetized cats (Neurosci. Abstr. 15:100, 1989) The purpose of the present study was to determine which subtype(s) of excitatory amino acid (EAA) receptor is(are) responsible for maintaining a normal breathing pattern at this site. For this purpose, antagonists of NMDA (3-((RS)-Carboxypiperazin-4-yl-)-propyl-1-phosphonic acid (CPP))and non-NMDA (6-Cyano-7-nitroquinoxaline-2,3-dione (CNQX)) receptors were microinjected bilaterally (50nl; pH 7.4) into this site (approx. 3mm rostral to obex, 4mm lateral to midline and 1.5mm below the ventral surface) while monitoring arterial blood pressure (BP), heart rate (HR), tidal volume (Vt) and respiratory rate (f) in chloralose anesthetized cats. In the case of CPP three doses were studied (0.25nmol, n=3; 0.75nmol, n=3; 2.25nmol, n=2). All three doses produced similar decreases in Vt (-12±1, p<0.05; -10±1, p<0.05, and -16±5mls respectively), and increases in f (+14±2, p<0.05; -10±3, p<0.05, and -12±3breaths/min respectively). None of these animals exhibited apnea. In contrast, microinjection of CNQX (0.05nmol, N=3) resulted in only a small increase in f (+1.7±0.3breaths/min; p< 0.05) with no significant change in Vt. A combination of CPP and CNQX (N=5) resulted in a large decrease in Vt (-24±7mls; p< 0.05) and an increase in f (+18±4breaths/min; p< 0.05). Three of the five animals tested exhibited apnea. None of these drugs produced significant effects on BP or HR. These results indicate that the major EAA drive to respiratory neurons in the caudal-subretrofacial area involves activation of NMDA receptors but excitation of non-NMDA receptors may also play a crucial role in maintaining respiration. Supported by USPHS grant 1P01 NS28130.

491.3

PHOSPHATE-ACTIVATED GLUTAMINASE (PAG) INHIBITORS ABOLISH GLUTAMATE-IMMUNOREACTIVITY IN THE RAT CEREBRAL CORTEX. F. Conti, M. Fabri, A. Minelli* and C. Sgattoni*. Institute of Human Physiology, University of Ancona School of Medicine, I-60131 Ancona (Italy).

Conversion of glutamine (Gln) to Glu catalised by phosphate-activated glutaminase (PAG) plays an important role in the formation of transmitter Glu. In the experiments reported here we have studied the effects of two PAG inhibitors -mersalyl acid (MA) and N-ethylmaleimide (NEM)- on the pattern of glutamate-immunoreactivity (IR) in the rat cerebral cortex.

MA (1mM) and NEM (1mM) were applied either intraparenchimally (0.2 µl) or topically (2 µl) to the parietal cortex. After 30 min. animals were perfused with 4% carbodiimide and the brain post-fixed for several days in 4% paraformaldehyde. Vibratome sections were then processed for Glu-immunocytochemistry (Conti et al. J. Neurosci., 7: 1887-1901, 1987). Controls included: sham injections, administration of physiological saline, and Nissl staining of adjacent sections to rule out aspecific effects, and MA and NEM applications into dorsal root ganglia (DRG) to verify whether the two compounds affected Substance P (SP) IR. Results can be summarized as follows: 1) both intraparenchimal and topical administration of MA and NEM results in a complete suppression of Glu-IR in the cerebral cortex; 2) this effect is not due to neuronal death, since Nissl staining of sections adjacent to those processed for Glu-immunocytochemistry reveals normal cortical architecture; 3) sham applications or injections of saline did not change the pattern of Glu-IR; 4) injections of both MA and NEM into DRG did not change the pattern of SP-IR.

Since PAG activity is involved in the formation of transmitter Glu, these results provide further evidence that Glu-IR observed in the cerebral cortex is related primarily to the transmitter pool of Glu.

491.4

RELEASE OF EXCITATORY AMINO ACIDS FROM PRIMARY AFFERENT FIBERS: AN *IN VITRO* TISSUE CULTURE STUDY

S. Jeftinija, K. Jeftinija *, Z. Korade, A. Larson, S.R. Skilling*, D. Smullin.
Dept. Vet. Anatomy, Iowa State University, Ames, Iowa 50011,
Dept. Vet. Biol., Univ. of Minnesota, St. Paul., MN 55108, USA.

Multiple lines of evidence implicate the excitatory amino acids, L-aspartate (L-Asp) and L-glutamate (L-Glu) as excitatory transmitters in the spinal cord. The specific objective of this study was to develop an experimental model to determine whether the EAA are released from primary afferents. Dorsal root ganglia (DRG) from 1 to 8-day-old rats were dissected and cultivated on chicken plasma-coated slides in Petri dishes for 7 to 10 days. After a 60 min equilibration period in Ringer's electrophysiological recording solution, the mean concentrations of EAA recovered during a 5 min interval were 1.24µM for L-Glu and 0.127µM for L-Asp. Stimulation of DRG organotypic cultures with either 25 or 50 mM potassium resulted in a concentration-dependent release of both L-Asp and L-Glu. Exposure of the cultures to 10µM capsaicin also caused release of both EAA. Peak increases of 84.14+12.75% (mean+SD) for L-Glu and 99.91+15.47% for L-Asp occurred within 10 min following exposure to high K^+. The stimulatory effect of K^+ was Ca^{2+}-dependent. Addition of high K^+ to cultures, from which DRG cell bodies, but not terminals were freshly removed, resulted in an increase of both EAA. High K^+, however, failed to increase the release of EAA from cultures where DRG cell bodies were removed 72 hours prior to release experiments. These results demonstrate that EAA are released from mammalian primary afferent fibers, and in particular, released from small diameter primary afferents. Work was supported by NIH grant NS27751, USDA grant PL95-113, NIDA grants DA 04190, DA 04090, DA00124.

491.5

ELECTRICAL-STIMULATION INDUCED RELEASE OF EXCITATORY AMINO ACIDS, INCLUDING SULPHUR-CONTAINING COMPOUNDS, FROM ACUTE HIPPOCAMPAL SLICES. J.M.Klancnik, M.Cuénod, B.H.Gähwiler and K.Q.Do Brain Research Inst., Univ. of Zürich, Switzerland.

Excitatory amino acids (EAAs) have been suggested as possible neurotransmitters in the hippocampus, and have been shown to be released by high-K^+ stimulation in a Ca^{2+}-dependent manner. To identify putative neurotransmitters released from specific synaptic locations, however, it is necessary to stimulate more selectively. The present study examined the release of amino acids from acute 400µm thick hippocampal slices prepared from adult SIV-50 rats upon electrical stimulation of Schaffer collaterals. In a submerged-type recording chamber, a 300µm diameter cannula was placed 50-60µm over the CA1 stratum radiatum, to collect 12 consecutive 1-min fractions of superfusate at a rate of 20µl/min. Electrical stimulation was applied via microelectrode for 4 min, at 50Hz, 100µsec pulse duration and an intensity which just evoked a population spike of maximal amplitude recorded in CA1 stratum pyramidale. Analysis was performed by reversed phase HPLC, following derivatization with o-phtalaldehyde, using a linear gradient of 0.03M acetate buffer (pH 7.0) and acetonitrile. Consistently, aspartate (Asp) levels were increased (from 0.05±0.03 to 1.55±0.71 pmol/fraction) upon stimulation, while levels of leucine, isoleucine, valine or phenylalanine showed no changes. An increase in glutamate levels was observed occasionally. In some experiments, low levels (approx. 0.2 pmol/fraction) of homocysteic acid (HCA) or cysteine sulfinic acid (CSA) were detected only during or following stimulation. Although high K^+-induced release of HCA and CSA from hippocampus has been shown previously, these results are the first to indicate a release of these sulphur-containing EAAs upon electrical stimulation. The present results suggest that Asp, HCA and CSA play a role in synaptic transmission in the Schaffer collaterals.

491.6

ARACHIDONIC ACID METABOLISM AND EXCITATORY AMINO ACID RELEASE. C.P. Duncan*, D. Martin and J.V. Nadler. Depts. Pharmacology and Neurobiology, Duke Univ. Med. Ctr., Durham, NC 27710.

It has been reported that metabolites of arachidonic acid are essential for catecholamine release from the adrenal medulla and stimulate glutamate release from synaptosomes. This study examined the effects of nordihydroguaiaretic acid (NDGA), an inhibitor of the lipoxygenase pathway, on the release of glutamate and aspartate from the Schaffer collateral-commissural-ipsilateral associational (SCCIA) pathway in area CA1 of the rat hippocampus. Transmitter release was evoked from superfused slices of the CA1 area (excluding stratum lacunosum-moleculare) by exposing the slices to 1-min pulses of 50 mM K^+. Under our conditions nearly all the release of glutamate and aspartate is Ca^{2+}-dependent and it originates predominantly from the SCCIA pathway.

Addition of 100 uM NDGA to the superfusion medium released endogenous glutamate and taurine from the slices. No aspartate release was detected. NDGA-evoked amino acid efflux was not Ca^{2+}-dependent. When the slices were depolarized with elevated K^+, NDGA depressed the evoked release of both glutamate and aspartate by about 80%. Indomethacin, an inhibitor of the cyclo-oxygenase pathway, did not affect glutamate or aspartate release.

These findings support a crucial role for lipoxygenase products of arachidonic acid metabolism in the synaptic release of excitatory amino acids. (Supported by NIH grant NS 16064.)

491.7

THE LOCALIZATION OF GLUTAMATE-METABOLIZING ENZYME mRNAs IN THE RAT CNS. K.M. Mearow, McMaster University, Hamilton, Ontario Canada

We have used in situ hybridization (ISH) to map the distribution of mRNAs coding for the glutamate-metabolizing enzymes, glutamine synthetase (GS), glutaminase (GA) and glutamate dehydrogenese (GDH) in the adult rat brain. ISH was carried out on frozen brain sections using synthetic 48-mer oligonucleotide probes directed against sequences coding for GS, GDH and GA.

GS mRNA was localized to glial cells in both white and grey matter. High density of labelling was associated with the Bergmann glial cell layer in the cerebellum, within the hippocampus and in certain cortical areas. GA mRNA was found to be enriched primarily in neurons. The location of labelled cells corresponded well with areas of known glutamatergic neurons - hippocampus, cerebellar granule layer, cortex (in particular, entorhinal cortex), pontine nuclei, olfactory bulb. GDH mRNA was found in both glial cells and neurons. The neuronal distribution of GDH was very similar to that of GA, while the glial distribution was similar to that of GS. The localization of the mRNAs corresponded well with the immunocytochemical distribution of cells labelled with antibodies against GS, GDH and GA.

491.8

ALTERATIONS IN REGIONAL KINETICS OF PHOSPHATE-ACTIVATED GLUTAMINASE WITH REGARDS TO AGE. D.R. Wallace and R. Dawson. University of Florida, Dept. Pharmacodynamics, Gainesville, FL. 32610.

Phosphate-activated glutaminase (PAG: L-glutamine amidohydrolase EC 3.5.1.2) hydrolyzes glutamine and thus forms both glutamate and ammonia. We have previously shown that a difference in regional ammonia inhibition exists between 8 month and 30 month old male Fischer-344 rats. The present study addresses the kinetics of PAG in the temporal cortex (TCX), striatum (STR), and hippocampus (HIPP) from 8 month and 30 month old F-344 rats. The basal activity (P2 fraction in the presence of 500uM glutamine and 10mM phosphate) was the highest in the TCX followed by the STR, and then the HIPP which had the lowest basal activity. Michaelis-Menten kinetics were done using substrate concentrations of 0.1-5.0mM glutamine in the presence of 10mM phosphate. The Km for PAG was unchanged in any of the regions from 8 month old rats. The values for Vmax displayed a similar trend as basal activity (TCX≥HIPP>STR). Vmax was significantly reduced approximately 15% in the STR with regards to age. In the HIPP, both Km and Vmax were significantly increased (approximately 30% and 25% respectively) with regards to age. There was no change in the kinetics of PAG in the TCX. The kinetic differences seen in this study and the changes seen in previous studies suggest that PAG is selectively altered in the aged brain, and that regional differences exist suggesting the existence of regional isozymes.
(Work supported by a predoctoral award from NIA (NO1-AG-3-2104) and a predoctoral fellowship (AG-00196-01) from the Center for the Neurobiology of Aging, Univ. of Florida.)

491.9

GLUTAMATE IMMUNOREACTIVITY IN TERMINALS OF CORTICAL EFFERENTS AND DORSAL ROOT AFFERENTS. R.J. Weinberg and A. Rustioni. Dept. of Cell Biology & Anatomy, University of North Carolina, Chapel Hill NC 27599.

Results obtained with techniques combining anterograde tracers with post-embedding electron microscopic immunocytochemistry will be illustrated using cortical efferents and primary afferents as models. Two days after injections of lectin-conjugated HRP in cortex, peripheral nerves, or dorsal root ganglia, rats were sacrificed by intraaortic perfusion with 2.5% glutaraldehyde, 0.5% para-formaldehyde, and 0.2% picric acid in phosphate buffer (pH 7.2). Peroxidase was demonstrated in 50 μm Vibratome sections of medulla and spinal cord, using a tungstate modification of the standard TMB histochemistry. DAB-stabilized sections were embedded in Epon-Spurr; thin sections on nickel grids were processed for post-embedding immunocytochemistry for glutamate and GABA using two different sizes of gold particles. Substantial numbers of glutamate- and GABA-labeled terminals were observed in both brain stem and spinal cord. Gold particles were concentrated over synaptic vesicles and mitochondria. Density of gold particles over anterogradely labeled terminals differed in terminals of different origins. The combination of these techniques with the retrograde tracer WGAapoHRP-Au, allows a fine-grain analysis of the chemical anatomy of neuronal microcircuitry.

491.10

GLUTAMATE AND ACETYLCHOLINE ARE CO-LOCALIZED IN THE LATERODORSAL TEGMENTAL AND PEDUNCULOPONTINE NUCLEI. J.R. Clements and S.J. Grant, School of Life and Health Sciences, Dept. Psychology and Prog. in Neuroscience, Univ. Delaware, Newark, DE. 19716.

Studies of the pedunculopontine (PPT) and laterodorsal tegmental (LDT) nuclei in the mesopontine tegmentum have emphasized the organization and projections of cholinergic neurons. Few studies have focused on the role of excitatory amino acids in these regions. Therefore, we decided to examine the distribution of glutamate-like immunoreactivity (GLI) in the rat mesopontine tegmentum.

GLI neurons were found extensively distributed throughout the mesopontine tegmentum. GLI neurons were intercalated among cholinergic neurons in the LDT and PPT, as well as in adjacent non-cholinergic areas. GLI neurons in the LDT and PPT were similar in morphological type and size to cholinergic neurons in these nuclei. Double labeling studies revealed a subpopulation of neurons in both the LDT and PPT which exhibited co-localization of GLI and ChA-T immunoreactivity. This suggests that some LDT and PPT neurons may contain two classical transmitters as well as previously described peptide co-transmitters.

These data raise the possibility that PPT and LDT target sites receive combined excitatory amino acid and cholinergic inputs and that excitatory amino acids contribute to the functions of the LDT and PPT, including behavioral state control, locomotion and activation of midbrain dopamine neurons.

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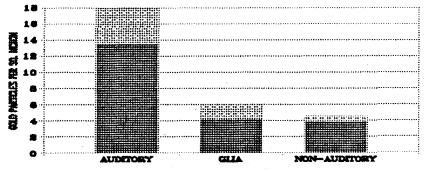
491.11

INCREASED LEVELS OF GLUTAMATE IMMUNOREACTIVITY IN THE AUDITORY NERVE ENDINGS OF THE DORSAL COCHLEAR NUCLEUS. J.M. Juiz, R.H. Helfert, R.J. Wenthold and R.A. Altschuler. Kresge Hearing Research Inst., Univ. Michigan, Ann Arbor, MI 48109 & LMO, NIDCD, NIH.

Increased levels of glutamate are likely to be associated with excitatory amino acid terminals, either as a transmitter or part of the transmitter pool. Quantitative evaluation of immunogold labeling provides a sensitive technique to determine glutamate levels in synaptic terminals. We have used this technique with glutamate antiserum in the cochlear nucleus, where auditory nerve terminals are believed to use an excitatory amino acid as their transmitter. The number of colloidal gold particles in auditory nerve terminals compared to other terminals and glial elements in the dorsal cochlear nucleus is seen see below:



The increased immunoreactive labeling for glutamate in auditory nerve terminals suggests that such labeling may serve as a useful marker for excitatory amino acid terminals.

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491.12

IMMUNOCYTOCHEMICAL LOCALIZATION OF N-ACETYLATED ALPHA-LINKED ACIDIC DIPEPTIDASE (NAALADase). B.Stauch Slusher, G. Tsai, and J.T. Coyle. Departments of Pharmacology and Neuroscience, The Johns Hopkins School of Medicine, Baltimore, MD 21205.

N-Acetylated alpha-linked acidic dipeptidase (NAALADase) is a membrane-bound metallopeptidase that cleaves glutamate from the endogenous neuropeptide N-acetyl-aspartyl-glutamate (NAAG). We have purified rat brain NAALADase activity to apparent homogeneity, and have raised specific anti-NAALADase antibodies in guinea pig (Stauch et al., *submitted*). Immunocytochemical studies show intense NAALADase-immunoreactivity (NAALADase-IR) in several structures previously reported to contain NAAG-immunoreactivity (NAAG-IR) including: the globus pallidus, entopeduncular nucleus, substantia nigra, hippocampus, reticular thalamic nuclei, medial and lateral geniculate, lateral habenular nucleus, periaqueductal gray, molecular and granular cell layers of the cerebellum, spinal trigeminal nucleus, substantia gelatinosa, lateral cervical nuclei, and intermediolateral cell column. Within these structures, immunoreactivity was distributed throughout the neuropil; no cell bodies were stained, even after colchicine treatment. The only exception was a subpopulation of cerebellar granule cells whose cell soma stained intensely. In addition, NAALADase-IR was observed in several fiber tracts, including the stria terminalis, fornix, solitary tract, and corpus callosum; these fiber tracts also contain NAAG-IR. The co-localization of NAALADase-IR and NAAG-IR within the same neural structures supports the hypothesis that NAALADase is responsible for the catabolism of NAAG *in vivo*.

491.13

Monoclonal Antibodies to N-Acetyl-Aspartate. M.L. Simmons*, C. Frondoza*, and J.T. Coyle. Dept. of Neuroscience, The Johns Hopkins University School of Medicine, Balto., MD 21205.

N-acetyl-aspartate (NAA) is found in high concentrations in all areas of the brain, but is undetectable in non-nervous tissue [Tallan, et al. 1956]. NAA and glutamate are produced as a result of the enzymatic degradation of N-acetyl-aspartyl-glutamate (NAAG), a putative neurotransmitter/neuromodulator [Robinson, et al. 1987]. However, current information suggests that NAA may have functions unrelated to NAAG. For instance, analysis by HPLC has shown that NAA is present at nearly ten times the concentration of NAAG, and is more homogeneously distributed than NAAG [Koller, et al. 1984]. Monoclonal antibodies to NAA were produced to further study its role in the brain.

Spleen lymphocytes from a mouse immunized with NAA conjugated to thyroglobulin by carbodiimide were fused with P3/x63-Ag8 mouse myeloma cells. Clones were produced which secrete IgG2a(K) antibodies specific for conjugated NAA. Seven percent cross-reactivity with conjugated NAAG was observed only at high antibody concentrations; no cross-reactivity was observed with conjugated N-acetyl-glutamate or aspartate. Preincubation of the antibody with 0.5 mg/ml conjugated NAA blocked immunoreactivity 97%, while preincubation with conjugated NAAG and free NAA had no effect. Preliminary immunocytochemistry has shown that NAA-immunoreactivity is localized in neurons and widely distributed throughout the brain. This monoclonal antibody exhibits a high degree of specificity for conjugated NAA and will be useful in elucidating the function of NAA in the brain.

491.14

ABNORMAL EXCITATORY NEUROTRANSMITTERS IN HUMAN AND CANINE DEGENERATIVE MOTO-NEURON DISEASES G. Tsai, L.C. Cork*, J.C. Hedreen, B. Stauch Slusher, L. Passani, J.D. Rothstein, and J.T. Coyle. Depts. Neuroscience, Psychiatry and Neurology, The Johns Hopkins School of Medicine, Balto., MD, 21205.

There is increasing evidence that excitatory neurotransmitters may be involved in the pathogenesis of degenerative motoneuron disease (DMD). Accordingly, we have measured aspartate (Asp), glutamate (Glu), N-acetylaspartate (NAA), and N-acetylaspartylglutamate (NAAG) in pathologically confirmed amyotrophic lateral sclerosis (ALS) and hereditary canine spinal muscular atrophy (HCMD). In punch samples of human ALS cervical cord (ALS n=8, control n=6), only the ventral horn exhibited significant decreases in Asp (-21%) and Glu (-17%). However, both NAA and NAAG were reduced substantially in ventral horn, dorsal horn, and ventral root (-40%, -44%, -50%; and -60%, -43%, -36% respectively). In motor cortex, Asp and NAAG decreased significantly in deep gray matter (-32% and -29% respectively). Asp (-48%) and Glu (-46%) were also decreased in the white matter. In the HCMD, Asp, Glu, NAA, and NAAG in the thoracic cord all decreased significantly (-53%, -55%, -23%, and -24% respectively), whereas NAA and NAAG were unchanged in the motor cortex. The motor cortex of the dogs is devoid of pathology which is limited to the lower motoneurons in the spinal cord. These findings support the excitotoxic hypothesis of DMD and are consistent with previous report of the elevated NAA and NAAG in human ALS CSF. Since NAA and NAAG are highly concentrated in motoneurons, they may play a role in the pathogenesis of DMD.

491.15

IMMUNOCYTOCHEMICAL CO-LOCALIZATION OF N-ACETYL-ASPARTATE AND N-ACETYLASPARTYLGLUTAMATE IN MONKEY. L. Passani, G. Tsai, B. Stauch Slusher, M. Simmons, J.T. Coyle. Depts. Neurosci. and Psychiat., Johns Hopkins Med. Sch., Balto, MD, 21205.

N-acetylaspartate (NAA) and N-acetylaspartylglutamate (NAAG), a putative neurotransmitter/modulator, are enriched in neuronal tissue. NAA has been suggested as a precursor and/or metabolite of NAAG. The development of affinity-purified antisera against conjugated NAAG has permitted the immunocytochemical investigation of its cellular localization in rodent nervous tissue. Now highly specific monoclonal antibodies against conjugated NAA has been successfully produced (see abstract, M. Simmons et al.). To further understand the neuroanatomical relationship between NAA and NAAG in primate, we have exploited a double-staining immunocytochemistry technique for visualizing these two antigens in the same sections of monkey brain. Six *Macaca mulatta* were perfused with a combination of paraformaldehyde and carbodiimide. Immunocytochemical specificity of the antibodies was demonstrated by their differential blocking by conjugated NAAG and NAA. Preliminary results indicate NAA and NAAG are co-localized in different structures of the monkey brains. Thus, neurons in motor and cingulate cortex, the nucleus of the diaganol band, septal nucleus, hippocampus, globus pallidus, substantia nigra, subthalamic nucleus, lateral geniculate, deep cerebellar nuclei, brain stem and spinal cord motor nuclei all contain NAAG and NAA immunoreactivity. However, NAA immunoreactivity was more widely distributed than that of NAAG. These results are consistent with previous findings on regional concentrations of NAA and NAAG. Also, they support a functional relationship between NAA and NAAG.

491.16

ISCHEMIC CHANGES OF EXCITATORY NEUROTRANSMITTERS IN RAT HIPPOCAMPUS. J.K. Deshpande, G. Tsai, L. Passani*, and B. Stauch. Dept. of Anesthesiology & Neurosciences, The Johns Hopkins Medical Institutions, Baltimore, MD 21205

Using in vivo microdialysis, we have previously demonstrated that aspartate (ASP), glutamate (GLU), and N-acetylaspartatylglutamate (NAAG) are released from rat hippocampus after global ischemia. To further understand the changes in the metabolism of excitatory neuromodulators after ischemia, we have determined the tissue content of ASP, GLU, and/or metabolic N-acetylaspartate (NAA), a precursor of NAAG and N-acetylated alpha-linked acidic dipeptidase (NAALDase), which changes NAAG to NAA and GLU immediately after global ischemic and then after 96 hr of recovery. Immunocytochemical staining with monoclonal antibodies specific for NAA and affinity-purified antisera for NAAG was performed in rats subject to transient ischemia and after 48, 72, and 96 hr of recovery. In this model, frank neuronal necrosis is evident only after 72 hr. Implications of these results in elucidating the role of NAAG in ischemic neuronal damage are discussed.

491.17

PREFERENTIAL ASTROGLIAL LOCALIZATION OF KYNURENINE AMINOTRANSFERASE IN THE RAT HIPPOCAMPUS. R. Schwarcz, F. Du, W. Schmidt and E. Okuno. Maryland Psychiatric Research Center, Baltimore, MD 21228.

Kynurenine aminotransferase (KAT), the biosynthetic enzyme of kynurenic acid, was recently purified to homogeneity from rat kidney, and its identity with brain KAT was established (Okuno et al., submitted). Using rabbit anti-rat KAT antibodies, we have now studied KAT immunohistochemically in the normal rat hippocampus.

Control experiments, including absorption of antibodies with pure enzyme, showed no immunoreactivity. KAT-immunoreactivity(-i) was predominantly observed in glial cells. In general, these cells had 4-6 primary processes radiating from the cell bodies. They were present in all hippocampal subfields along the rostrocaudal axis. The hilus contained a higher density of KAT-i glial cells than CA1-3 regions, whereas the granule cell layer and the adjacent portion of the molecular layer of the dentate gyrus harbored only a few KAT-i glial cells. As demonstrated by double-labeling with glial fibrillary acidic protein, the vast majority of KAT-i cells appeared to be astrocytes. In addition, sporadic neurons containing KAT-i were also detected, mainly in strata oriens and pyramidale. These neurons probably belong to a subpopulation of interneurons.

The organization of cellular elements containing KAT might be of relevance for the function of kynurenic acid in the hippocampus. (Supported by USPHS grant NS 16102).

491.18

IMMUNOHISTOCHEMICAL LOCALIZATION OF QUINOLINIC ACID PHOSPHORIBOSYLTRANSFERASE IN THE HUMAN NEOSTRIATUM. C.Köhler, F. Du[1], E. Okuno[1], W.O. Whetsell, Jr.[2], and R. Schwarcz[1]. ASTRA Research Center, Södertälje, Sweden, [1]Md. Psych. Res. Ctr., Baltimore, MD 21228, and [2]Dept. Pathology, Vanderbilt Univ., Nashville, TN 37232.

The localization of quinolinic acid phosphoribosyltransferase (QPRT), the degradative enzyme of the endogenous excitotoxin quinolinic acid, was studied in the human neostriatum by immunohistochemistry. In 8 neurologically normal human brains, QPRT-immunoreactivity (QPRT-i) was detected in both glial cells and neurons. Glial cells containing QPRT-i had numerous processes radiating from the cell bodies. In Nissl-counterstained sections, most QPRT-i glial cells showed morphological features of astrocytes. Neurons containing QPRT-i had different sizes and shapes and were tentatively classified into three subpopulations. Most were medium-sized cells with ovoid or elongated perikarya. Small QPRT-i neurons, often spheroid in shape, were particularly noted in a zone of the caudate nucleus adjacent to the lateral ventricle. A few large QPRT-positive neurons were also observed. The somatic and dendritic morphology of QPRT-i neurons resembled that of aspiny neurons seen in Golgi preparations.

The localization of QPRT in distinct populations of neostriatal cells suggests specific functional correlates, and may be relevant for the pathogenesis of basal ganglia disorders. (Supported by USPHS grant NS 28236).

491.19

ANALYSIS OF THE DISTRIBUTION OF A PUTATIVE KAINIC ACID RECEPTOR IN MAMMALIAN BRAIN USING AN ANTI-KAINIC ACID RECEPTOR MONOCLONAL ANTIBODY. J.W. Goh[3], X.-P. Huang*[1], A. Auyeung*[1], C.J. Dechesne[2], R.J. Wenthold[2], M.D. Oberdorfer[2]and D.R. Hampson[1]. [1]Faculty of Pharmacy, University of Toronto, Toronto, Ontario, M5S 2S2; Laboratory of Molecular Otology, National Institutes of Health, Bethesda, MD 20892; [3]Dept. of Pharmacology & Toxicology, Queen's University, Kingston, Ontario, K7L 3N6.

Kainic acid and AMPA receptors mediate fast excitatory synaptic transmission in the CNS. Although the distribution of kainic acid receptors has been studied in a number of species using receptor autoradiography, very little is known about their cellular and subcellular distribution. We have produced a monoclonal antibody against a kainate protein purified from frog brain. This antibody recognizes a protein in rat and mouse that is brain-specific and has a Mr = 99,000. Immunoblot analysis of mouse brain revealed that the Mr = 99,000 protein was not detectable in the hippocampus until postnatal day 7; in the cerebellum, immunoreactivity was not observed at postnatal day 7 but was very intense in adult tissue. The subcellular distribution of this protein was examined by immunoblot analysis of purified subcellular fractions prepared from rat forebrain. The protein was not present in the soluble or myelin fraction but was present in mitochondrial and microsomal membranes and was highly concentrated in synaptic membranes and synaptic junctional fractions. Lesion studies in which domoic acid was microinjected into the amygdala resulted in a decrease in immunostaining in the hippocampus 30 days after injection. These results indicate that this monoclonal antibody recognizes a kainic acid receptor with a Mr = 99,000 and that this receptor is located postsynaptically on nerve cell membranes. Immunocytochemistry at the EM level confirmed these results.

Supported by NSERC and MRC (Canada).

491.20

ONTOGENY OF EXCITATORY AMINO ACID RECEPTORS IN RAT BRAIN. R.L. Makowiec, S.Y. Sakurai, J.B. Penney, and A.B. Young. Dept. of Neurology and Neuroscience Program, University of Michigan, Ann Arbor, MI, 48109.

The ontogenetic profile and regional distribution of excitatory amino acid (EAA) receptors were examined using quantitative autoradiography. We measured binding in the outer cortex, striatum, globus pallidus (GP), CA1, CA3, and dentate gyrus (DG) in 1-, 4-, 7-, 10-, and 14-day-old male rat pups. The NMDA receptor complex was labelled using [^{3}H]MK-801, [^{3}H]glycine and NMDA-sensitive [^{3}H]glutamate binding. Ionotropic and metabotropic quisqualate receptors were examined using [^{3}H]AMPA and AMPA-insensitive, quisqualate-sensitive [^{3}H]glutamate binding (AiQsGB), respectively.

From postnatal day (PND) 1 to PND 14 [^{3}H]MK-801 and [^{3}H]glycine binding increased in CA1, outer cortex and striatum while in GP binding remained at low levels. NMDA-sensitive [^{3}H]glutamate binding exhibited a different profile. In striatum, CA1 and CA3 binding peaked at PND 7 and remained constant to PND 14. Binding in GP decreased from PND 1 to PND 14. These data suggest that the various components of the NMDA receptor may be differentially expressed during development.

[^{3}H]AMPA binding increased from PND 1 to PND 14 in all regions examined except in CA3 where binding peaked at PND 7 and remained constant. Metabotropic binding (AiQsGB) peaked at PND 10 in striatum and outer cortex and then decreased at PND 14. In CA1 and DG, metabotropic binding increased during the first two weeks. In CA3, however, binding peaked at PND 7 and then decreased over the next week. Interestingly, in GP the relative amount of metabotropic binding was high at PND 1 and remained at this level through PND 14. These results suggest EAA receptors have unique ontogenetic profiles and may be involved in a variety of developmental CNS processes. Supported by USPHS Grant NS 19613.

491.21

EXCITATORY AND INHIBITORY AMINO ACID RECEPTORS IN HUMAN STRIATE CORTEX. W.F. Maragos, R.L. Albin, S.Y. Sakurai, R.L. Makowiec, D.S. Higgins, A. B. Young and J.B. Penney. Dept. of Neurology and Neuroscience Program, University of Michigan, Ann Arbor, MI. 48104.

Quantitative receptor autoradiography was used to determine the distribution of excitatory amino acid and inhibitory amino acid receptors in four human striate cortex specimens. Cytoarchitecture was determined by Nissl and cytochrome oxidase staining. $GABA_A$ receptors were densest in Layers 2-3 and 4C, intermediate in 4B, and lowest in Layers 1 and 5-6. $GABA_B$ receptors had a much lower density in Layers 1-3, intermediate in 4C-6 and lowest in 4B. NMDA receptors were densest in Layers 2-3 and 4C, intermediate in 1 and 4B and lowest in 5-6. Kainate receptors were densest in Layers 1 and 5-6. AMPA receptors were densest in Layers 1-3, intermediate in 5-6 and lowest in 4B-4C. Metabotropic receptors were densest in Layers 1-4A, intermediate in 4B and 5-6 and lowest in 4C. Distinct differences in receptor distribution were noted between areas 17 and 18. Supported by USPHS Grant NS 01300 and AG 06155.

491.22

DECREASED QUISQUALATE-SENSITIVE GLUTAMATE BINDING IN THE CEREBELLAR CORTEX OF THE DYSTONIC RAT. M.W. O'Brien, G.A. Oltmans, and J.F. Lorden. Dept. of Pharm. and Mol. Biol., Chicago Med. Sch., N. Chicago, IL 60064 and Dept. of Psych., Univ. of Alabama, Birmingham, AL 35294.

The genetically dystonic rat (*dt*) exhibits a complex and progressive movement disorder initially detectable on post-natal days 9 or 10. No detectable morphologic defects of the nervous system are apparent. The mutant is also insensitive to the tremorogenic effects of the drug harmaline. Recording studies suggest this insensitivity is linked to a defect in the olivo-cerebellar pathway, since few Purkinje cells of the *dt* rat show the typical increase in complex spike activity following harmaline. Recent studies indicate the failure to elicit harmaline-tremor is not due to a failure to activate olivary neurons. Thus the defect in the olivo-cerebellar pathway may, in part, be due to faulty climbing fiber - Purkinje cell (CF-PC) interaction. This study was designed to determine if an abnormality in cerebellar excitatory amino acid (EAA) receptors might contribute to the failure to elicit harmaline tremor.

Serial cerebellar slices from 16-d old *dt* rats were incubated in [3H]glutamate with or without competitors in the presence or absence of 2.5 mM $CaCl_2$. Two-way Analysis of Variance revealed a significant decrease in $CaCl_2$-independent total glutamate binding and quisqualate-sensitive glutamate binding in the cerebellar cortex ($p < 0.05$). Alterations in $CaCl_2$-dependant total and quisqualate-sensitive binding were not statistically significant ($p = 0.12$ and $p = 0.08$ respectively). Neither N-methyl-D-aspartate- nor kainate-sensitive binding appeared to be altered. These data suggest that altered cerebellar quisqualate receptors may contribute to an abnormality in CF-PC communication in the *dt* rat. (Supported in part by the Dystonia Medical Research Found.)

BEHAVIORAL PHARMACOLOGY: OPIATES, NMDA AND OTHERS

492.1

ANALYSIS OF THE DISCRIMINATIVE STIMULUS EFFECTS OF 3,4-METHYLENEDIOXYMETHAMPHETAMINE (MDMA). J. Broadbent, C. A. Metosh, * E. E. Riddle * and J. B. Appel. *Behavioral Pharmacology Laboratory, Department of Psychology, University of South Carolina, Columbia, SC 29208.*

It has been shown that the (+) and (-) isomers of 3,4-methylenedioxamphetamine (MDA) have discriminably different behavioral effects; e. g., (-) MDA is more similar to hallucinogens such as (+) lysergic acid diethylamide (LSD) and (±) 2,5-dimethoxy-4-methylamphetamine (DOM) and is probably more serotonergic than (+) MDA. The extent to which similar effects occur with the isomers of MDMA ("ecstasy"), the n-methyl derivative of MDA, has not yet been clearly established. Rats were therefore trained to discriminate either (+) MDMA (1.25 mg/kg), (+) MDA (1.25 mg/kg), (-) MDA (1.25 mg/kg), (+) amphetamine (1 mg/kg), or LSD (0.16 mg/kg) from saline and were tested with both isomers of MDMA and several related drugs of abuse. Neither (+) nor (-) MDMA resembled (+) amphetamine, cocaine, LSD, DOM or mescaline, independent of the training compound. The 5-HT antagonist pirenpirone did not significantly affect the stimulus properties of (+) MDMA. However, the isomers of MDA and MDMA cross-generalized and, in each instance, the (-) enantiomer was less potent than the (+) enantiomer. These data suggest that the subjective effects of (+) and (-) MDMA are unlike those of stimulants and hallucinogens but do resemble those of MDA.

Supported by **USPHS Research Grant R02 DA02543**, from the *National Institute on Drug Abuse*.

492.2

WITHDRAWN

492.3

BEHAVIORAL EFFECTS OF SIGMA LIGANDS: CORRELATION WITH SIGMA AND PCP RECEPTOR AFFINITY. J.G. Wettstein, F.J. Roman* and J.L. Junien*. Institut de Recherche Jouveinal, 3-9 Rue de la Loge, 94265 Fresnes, France.

Drugs thought to have affinity for sigma sites were studied for their effects under a fixed-ratio schedule of food reinforcement in rats. Dose-related decreases in FR responding were observed with each drug. Rimcazole, +PPP and haloperidol were studied for their effects as antagonists of the rate-decreasing effects of JO 1784 (+N-cyclopropylmethyl-N-methyl-1,4-diphenyl-1-ethylbut-3-en-1-ylamine), DTG and +NANM: haloperidol did not attenuate the rate-decreasing effects of JO 1784, DTG and +NANM; rimcazole attenuated the effects of +NANM but not JO 1784; and +PPP attenuated the effects of +NANM but not JO 1784 and DTG. Moreover, BMY 14802 did not antagonize the rate-decreasing effects of +PPP. Drugs were then examined for their capacity to inhibit (3H)DTG, (3H)+PPP, (3H)+NANM and (3H)TCP receptor binding in rat whole brain membrane preparations. For all drugs, the binding IC50's for the 4 radioligands were compared with the doses required to decrease FR responding to 50% of the control rate. These results indicate that in spite of weak correlation coefficients the potencies of the drugs in decreasing FR rate correlated better with their affinities for (3H)DTG and (3H)+PPP sites than for (3H)+NANM and (3H)TCP sites, however, it was difficult to distinguish between purported sigma agonists and antagonists using FR behavior in rats.

492.4

INFLUENCE OF SIGMA LIGANDS ON APOMORPHINE-INDUCED CLIMBING IN MICE. L.R. Moser*, M.A. Krizek*, D.J. McGinness Grimes*, N.L. Katz and R.F. Schlemmer, Jr. Dept. of Pharmacodynamics, University of Illinois at Chicago, Chicago, IL 60612.

The role of sigma sites in the CNS in the mediation of behavior has not been clearly elucidated. One interesting observation is that the sigma ligands BMY14802 (BMY) and rimcazole (RIM) antagonize apomorphine (APO)-induced climbing in mice (Taylor et al, Soc Neurosci Abstr 11:114, 1985; Ferris et al., J Pharm Pharmacol 34:388, 1982), a dopamine-mediated behavior. The purpose of the present study was to test the effect of four sigma ligands that do not bind to dopamine receptors - ditolylguanidine (DTG),(+)3-(3-hydroxylphenyl)-N-(1-propyl)piperidine([+]3-PPP), RIM and BMY - on APO-induced climbing in mice. Male Swiss albino mice (23-38 g) were treated with DTG (1-10 mg/kg) s.c. and APO (0.3-2 mg/kg) s.c. to observe dose-dependent changes for each drug. The effect of DTG (3 mg/kg) was also tested on the dose-dependent changes induced by APO (0.3-2 mg/kg). RIM (1-50 mg/kg), (+)3-PPP (1-30 mg/kg) and BMY (3-30 mg/kg) were given s.c. 30 min. prior to APO (1 mg/kg) s.c. In each experiment, mice were observed every 2 min. for the presence or absence of climbing for 30 min. after APO treatment. APO-induced dose-dependent increases in climbing, but DTG did not. Moreover, DTG did not alter APO-induced changes in climbing. BMY significantly decreased APO-induced climbing in a dose-dependent manner. RIM inhibited APO climbing; however, at effective doses (≥20 mg/kg) tremors and seizures were noted. (+)3-PPP did not alter APO-induced climbing. This study replicated previous data for BMY but failed to extend these findings to two additional sigma ligands (+)3-PPP and DTG. Although RIM antagonized APO climbing, it is not possible to clearly determine if this effect was specific or due to the presence of movement abnormalities. The results do not provide conclusive evidence for a role for sigma mediation of APO-induced climbing behavior in mice.

492.5

MU AND DELTA OPIOID AGONISTS QUIET ISOLATION-INDUCED VOCALIZATIONS IN RAT PUPS. **Susan E. Carden, Gordon A. Barr, Myron A. Hofer. Columbia University, Physicians and Surgeons.**

During the first three weeks of life, rat pups react to separation from the homecage, dam, and littermates by emitting ultrasonic vocalizations. In 10-day olds, these characteristic distress cries are diminished if either a social companion or morphine (0.125mg/kg) are provided. The opioid antagonist naltrexone reverses the quieting effects of a littermate or dam, as well as blocking morphine effects, suggesting a role for opioids in the modulation of early isolation distress.

Isolated 10-day old rat pups received intracisternal injections of agonists specific to the mu, delta, or kappa opioid recectors, (DAGO (0.001ug - 0.0625ug), DPDPE (0.3ug - 3.0ug) and U50-488 (1.0ug - 100ug). The rate of vocalization was monitored for 6 minutes. Pups were evaluated for sedation and each injection site was verified. Mu and delta agonists DAGO and DPDPE decreased the number of distress cries in non-sedated animals, while the kappa agonist U50-488 left vocalization rates unchanged.

492.6

ENHANCED SENSITIVITY TO NALTREXONE IN RATS: EFFECTS ON SALIVATION AND OPIOID RECEPTOR BINDING. C.W. Schindler, X.-Z. Wu*, T.-P. Su, E.B. Thorndike*, S.R. Goldberg* and J.L. Katz. NIDA Addiction Research Center, Baltimore, MD 21224.

Previous research (JPET 252: 8-14) showed that rats develop enhanced sensitivity to the effects of naltrexone (NAL) on operant responding when it is given in a cumulative dosing manner once per week. Enhanced sensitivity was also observed to the salivation produced by NAL. The purpose of the present experiment was to determine if the enhanced sensitivity observed for salivation would also occur independent of operant responding, and to determine whether any changes in opioid receptor binding would be observed following the regimen of NAL treatment. Rats were treated with NAL once weekly in a cumulative dosing procedure. The rats were initially given an i.p. injection of 1 mg/kg NAL followed 13 min later by a 2 mg/kg injection and so on until five injections had been given (1, 2, 7, 20 and 70 mg/kg) for total cumulative doses of 1, 3, 10, 30 and 100 mg/kg. Salivation was scored on a 3 point scale. Initially, no salivation was observed at any dose; however, over the course of an 8-week period, increasing amounts of salivation were observed at the 10, 30 and 100 mg/kg doses. This effect was long-lasting. After a period of 10 weeks during which no NAL was given, NAL produced similar degrees of salivation as after week 8. Animals injected with saline for 8 weeks showed no salivation and saline given to the animals which had received NAL had no effect. As with the enhanced sensitivity observed on operant responding, the salivation appeared to extinguish to lower doses when the higher doses of NAL were omitted. A separate group of 6 rats was treated with NAL for 8 weeks as above. One week following the last NAL treatment, their brains were removed and assayed for opioid receptors. In comparison to animals that were treated with saline for 8 weeks, mu receptors were unchanged in all brain areas assayed. Kappa and delta receptors were increased in an area containing primarily cortex, and delta receptors were decreased in midbrain. These results indicate that enhanced sensitivity to NAL can develop independently of operant responding and is correlated with some changes in opioid receptors.

492.7

DIPRENORPHINE DRUG DISCRIMINATION: ASSESSMENT OF NALTREXONE AND NALOXONE SUBSTITUTION. S. Smurthwaite and A.L. Riley. The American University, Washington, D.C. 20016.

DeRossett and Holtzman (J. Pharmac. Exp. Ther., 237: 437-444, 1986) have recently reported that monkeys were able to discriminate between the opiate antagonist diprenorphine HCl and its vehicle. However, other opiate antagonists failed to substitute for the diprenorphine cue. Paradoxically, opiate agonists did substitute for diprenorphine. Because Geter and her colleagues (Neurosci. Abst., 15:248, 1989) have recently demonstrated generalization between diprenorphine and naloxone in naloxone-trained subjects within a taste aversion procedure, the present experiment reexamined drug discrimination learning with diprenorphine within this design. Specifically, every fourth day for 13 conditioning trials rats were administered diprenorphine (3.2 mg/kg) 15 min prior to a saccharin-LiCl pairing. On intervening days, they received the diprenorphine vehicle 15 min prior to saccharin alone. Following acquisition of the discrimination, both naltrexone (0.18 to 10 mg/kg) and naloxone (0.32 to 32 mg/kg) substituted for diprenorphine, while the opiate agonist morphine (3.2 to 10 mg/kg) did not. The present data are consistent with other work within the taste aversion design demonstrating generalization between the opiate antagonists.

492.8

CHARACTERIZATION OF A NOVEL CLASS OF POTENT AND SELECTIVE SIGMA LIGANDS. P. C. Contreras, M. Bremer, L. Christine *, S. Iyengar, V. Dilworth, B. Cheng* and N. M. Gray*. CNSDR, G. D. Searle & Co., St. Louis, MO 63198.

Much less is known about sigma than phencyclidine (PCP) receptors because there are very few selective sigma ligands. The purpose of this study was to characterize the biological activity of a novel class of sigma ligands, 9,10-ethanoanthracene-like compounds and related analogs, which interacted selectively with sigma, but not PCP receptors. SC-48960 and SC-49574, two of the more potent sigma ligands in this series, had very weak affinity for $alpha_1$-, $alpha_2$-, $beta_2$-adrenergic or $dopamine_1$ receptors. SC-48960 had moderate affinity for muscarinic, $serotonin_2$ and $dopamine_2$ receptors, but SC-49574 had no affinity for these receptors. SC-48960 and SC-49574 did not produce any stereotyped behavior or ataxia in rats, but antagonized (+)SKF-10,047-induced stereotyped behavior and ataxia. These results indicate that SC-48960 and SC-49574 are potent and selective sigma ligands capable of antagonizing some of the behavioral effects of sigma ligands.

492.9

CENTRAL ADMINISTRATION OF ß-CASOMORPHINS AFFECT ANTINOCICEPTIVE BEHAVIOR IN NEONATAL RATS. J.M.C. Blom*, E.M. Blass and S. Hulse. Dept. of Psychology, Johns Hopkins University, Baltimore, MD 21218.

ß-casomorphins (ß-CMs) represent a group of opioid peptides derived from the enzymatic digests of a milk protein (ß-casein). ß-CMs are an interesting class of substances because they provide a means through which mother's milk may affect infants, possibly through an opioid pathway. A number of experiments in adult rats have demonstrated antinociception after central administration of ß-CM.

In the present studies (Exp. 1) we evaluated in day 10 rats the antinociceptive potencies of ß-CM injected intracerebroventricularly (ICV). In Exp. 2, by centrally injecting naloxone, we evaluated whether the antinociceptive effects of ß-CM were opioid mediated.

In Exp. 1, 10-day-old rat pups were injected ICV either with .25 Ug ß-casomorphin-5 (TYR-PRO-PHE-PRO-GLY), .25 Ug morphine (for comparison) or isotonic saline, in a volume of 1 Ul. Pain responsivity was assessed 20, 40 and 60 min. post injection by placing the rats left forepaw on a hotplate (48°C). Paw lift latency (PLL) increased significantly for pups injected with ß-CM and morphine when compared with saline injected pups. Increased PLL lasted for at least 60 min. In Exp. 2 pups received an ICV injection of either .25 Ug naloxone or isotonic saline, followed immediatly by an IP injection of either .5 mg/kg ß-CM-5, .1 mg/kg morphine or isotonic saline. ICV pretreatment with naloxone blocked the decrease in heat nociception caused by ß-CM-5 to a level comparable to that of saline.

These results demonstrate that ß-CM can influence antinociceptive behavior in infant rats through a central mechanism that seems to be opioid mediated.

492.10

DRUG-DRUG DISCRIMINATION WITH THE OPIATE ANTAGONISTS NALOXONE AND DIPRENORPHINE. M.A. Kautz and A.L. Riley. The American University, D.C. 20016.

Recently, it has been reported that diprenorphine substitutes for naloxone in subjects trained to discriminate naloxone from distilled water (Geter et al., Neurosci. Abstr., 15:248, 1989), suggesting that there are similarities between the stimulus properties of the two drugs. Given that the two drugs have differential affinities for the various subtypes of the opiate receptor and that animals can differentiate between opiate agonists that differ in this regard, the present study attempted to train animals to discriminate between naloxone and diprenorphine. Specifically, rats with a history of discriminating naloxone from distilled water in a conditioned taste aversion/drug discrimination procedure were given naloxone prior to a saccharin-LiCl pairing and diprenorphine prior to saccharin alone. Although diprenorphine initially substituted for naloxone, with repeated training subjects acquired the discrimination. In subsequent generalization tests, the substitution was selective with naltrexone generalizing to naloxone and nalorphine generalizing to diprenorphine. The discrimination and the subsequent substitution profiles are consistent with their differential receptor activity.

492.11

THE ANTAGONISM OF THE MORPHINE STIMULUS IN DRUG DISCRIMINATION LEARNING: AN ASSESSMENT WITHIN THE TASTE AVERSION DESIGN. S. Pournaghash, G. Stevenson* and A. L. Riley. The American University, Washington, D. C. 20016.

Putative antagonists can be given concurrent with the training drug in a drug discrimination learning procedure (DDL) to assess the ability of the antagonist to block the stimulus properties of the compound. That DDL within the taste aversion design is so rapidly acquired and robust may preclude this antagonism. This possible limitation was addressed in the present experiment. Following the establishment of a morphine discrimination (5.6 mg/kg), naloxone (1 mg/kg) was given either 10, 30, 60 or 180 min prior to the administration of morphine. When naloxone preceded morphine administration by 10 and 30 min, it completely antagonized the discriminative effects of morphine. By 60 min, naloxone no longer antagonized the discriminative effects. That naloxone blocked the discriminative effects of morphine at these intervals is consistent with other work on the interaction of naloxone and morphine in more traditional DDL designs and indicates that the rapid acquisition of DDL within the taste aversion procedure does not necessarily preclude its utility in assessing drug antagonism.

492.12

BEHAVIORAL EFFECTS OF THE NON-COMPETITIVE NMDA ANTAGONIST MK801 IN INFANT AND PREWEANLING RAT PUPS. L. Rajachandran, G. Goodwin and L.P. Spear, Dept. of Psychology and Center for Developmental Psychobiology, SUNY Binghamton, NY 13901.

Neonatal (3-4 day old) and preweanling (17-18 day old) Sprague Dawley rat pups were tested following the administration of (0) saline, .01, .05, .5, .1 and 1mg/kg MK801 s.c. Pups were observed in a premilk/milk test situation 30 and 60 minutes post injection. Behaviors were sampled for 5 seconds every 20 sec. for 5 minutes in each condition. In neonatal rat pups, reductions in a number of behaviors (forward locomotion, mouthing) were seen at the higher (.5 and 1mg/kg) doses. In contrast, evidence of behavioral stimulation was seen at a lower dose (.1mg/kg) in forward locomotion at 30 min. post injection. In preweanling rat pups, marked sedative effects of MK801 were seen at higher doses (decreases in forward locomotion, headlift and sniff) with signs of behavioral stimulation (increases in forward locomotion and mouthing) evident at low doses. Thus, as in adults, low doses of MK801 are behaviorally stimulatory and higher doses inhibitory to both neonatal and preweanling pups, although these effects are somewhat more pronounced in preweanling than neonatal rat pups. Investigation of the behavioral consequences of NMDA antagonists such as MK801 is important, given the possible therapeutic potential of these substances for use in young organisms.

492.13

ANTIPUNISHMENT EFFECTS OF NPC 12626 DURING ACUTE AND REPEATED DOSING IN RATS. **J.L. Wiley, J.H. Porter, A.D. Compton, and R.L. Balster. Depts. of Psych. and Pharm./Tox., VA Commonwealth Univ., Richmond, VA 23284.**

Rats were tested in a modified Geller-Seifter (1960) conflict test (MULT FI 60-sec FR 1) to determine if the competitive NMDA antagonist, 2-amino-4,5-(1,2-cyclohexyl)-7-phosphonoheptanoic acid (NPC 12626), would produce an increase in punished responding during acute and repeated dosing. Chlordiazepoxide (CDP) and phencyclidine (PCP) also were tested.

During separate acute dose determinations, NPC 12626 (30 and 56 mg/kg), CDP (10 mg/kg), and PCP (4 mg/kg) produced selective increases in punished responding relative to vehicle. During a six day repeated dosing procedure, the 30 mg/kg dose of NPC 12626 produced a selective increase in punished responding during 5 of the 6 days. PCP (2 mg/kg) produced a selective increase in punished responding on 3 of the 6 days; whereas, the 5 mg/kg dose of CDP produced significant increases in both punished and unpunished responding on 3 of the 6 days. These results demonstrate that the competitive NMDA antagonist NPC 12626 has selective antipunishment effects in an antianxiety animal model.

492.14

CHRONIC TREATMENT WITH MK801 PRODUCES TOLERANCE TO THE BEHAVIORAL EFFECTS. L.M. Ford, P.R. Sanberg, S.R. King*, and A.B. Norman. Division of Neuroscience, Departments of Psychiatry and Neurology, University of Cincinnati College of Medicine, Cincinnati, OH 45267.

Acute systemic injection of MK801, a non-competitive N-methyl-D-aspartate (NMDA) receptor antagonist induces locomotor hyperactivity, decreased rearing behavior and increased stereotypy (Ford et al., Physiol. and Behav. 46:755-758, 1989). Following chronic treatment with a number of receptor antagonists a tolerance to the behavioral effects is observed. We therefore compared the topography of MK801-induced locomotor activity after acute and chronic treatment to evaluate the possibility of tolerance or sensitization. Rats (n=8) received 0.5 mg/kg i.p. once daily for 21 days; locomotor activity was measured after the 1st (acute) and 21st (chronic) injection using the Digiscan Animal Activity Monitor. The increased ambulation (horizontal activity) was not significantly different between acute and chronic MK801 treatments. Rearing behavior was reduced following acute MK801 treatment compared to saline treated controls ($p<0.05$). However, following 21 days of treatment these MK801-induced reductions in rearing behavior were reversed and in some cases were elevated above control values. Thigmotaxis (wall hugging behavior), decreased with chronic treatment ($p<0.05$). Thus, chronic treatment with MK801 produced adaptations in the topography of locomotor activity of rats representative of tolerance to the behavioral actions of MK801. It is possible that neurochemical adaptations may underlie these adaptations in behavior. Supported by University of Cincinnati Research Council, Childrens Hospital (Cincinnati) and NINDS.

492.15

USE OF THE PIGEON CONFLICT PROCEDURE TO CHARACTERIZE ANXIOLYTIC DRUG ACTIVITY: EVALUATION OF N-METHYL-D-ASPARTATE ANTAGONISTS. W. Koek*, M.J. Brocco*, J.C.R. Randle and F.C. Colpaert*. FONDAX - Groupe de Recherche SERVIER, 7 rue Ampère, 92800 Puteaux, FRANCE.

The antipunishment effects of the clinically effective anxiolytics chlordiazepoxide (CDP), buspirone and ritanserin were evaluated using conflict procedures in rats (Vogel, Geller-Seifter) and in pigeons (Barrett). In rats, only CDP was found to have significant antipunishment activity. In pigeons, however, antipunishment effects were produced not only by CDP, but also by buspirone and ritanserin.

Because of its apparent effectiveness in detecting non-benzodiazepine anxiolytic agents, the pigeon conflict procedure was used to evaluate possible antipunishment activity of various N-methyl-d-aspartate antagonists. Punished responding was significantly increased by competitive NMDA antagonists (i.e., CPP and CGS 19755), but not by non-competitive NMDA antagonists acting at the NMDA receptor-associated ion channel (i.e., phencyclidine, ketamine and MK-801). In preliminary experiments, non-competitive NMDA antagonists thought to act at the NMDA receptor-associated glycine site (e.g., kynurenic acid, 7-chloro-kynurenic acid) failed to show significant antipunishment effects.

492.16

INDOMETHACIN ATTENUATION OF RADIATION-INDUCED HYPERTHERMIA DOES NOT AFFECT RADIATION-INDUCED HYPOACTIVITY. J.L. Ferguson, S.B. Kandasamy, A.H. Harris, M.R. Landauer and H.D. Davis. Dept of Behavioral Science, AFRRI, Bethesda, MD 20814-5145.

Exposure of rats to 5-10 Gy of high-energy electrons produces hyperthermia and reduces motor activity. Previous studies using a 60Co source suggest that radiation-induced hyperthermia results from a relatively direct action on the brain and is mediated by prostaglandins. To test the hypothesis that hypoactivity may be, in part, a thermoregulatory response to this elevation in body temperature, adult male rats were given indomethacin (0.0, 0.5, 1.0, 3.0 mg/kg, I.P.) and either irradiated (LINAC 18.6 MeV high-energy electrons, 10 Gy at 10 Gy/min, 2.8 usec pulses at 2 Hz) or sham-irradiated. The activity of all rats was then measured for 60 min in a photocell monitor for distance travelled and number of vertical movements. Rectal temperatures of irradiated rats given only vehicle were elevated by 0.9-1.1°C at both the beginning and end of the activity session. Indomethacin, at any dose, attenuated radiation-induced hyperthermia. Irradiated rats showed less motor activity than sham-irradiated rats. The decreased motor activity of the irradiated rats was not affected by any dose of indomethacin. These results suggest that the increased body temperature following irradiation is probably not a cause for the decreased motor activity.

492.17

GENETIC DIFFERENCES IN CONDITIONED TASTE AVERSION. N. E. Colley[1] and A. C. Collins [1,2]. [1]Psychology Department and [2]Institute for Behavioral Genetics, University of Colorado, Boulder, CO 80309.

Variability exists in the acquisition of illness-induced consumatory aversions. In an attempt to ascertain if genetic factors contribute to this variability, male mice from 6 inbred strains (A/J/Ibg, C3H/2Ibg, C57BL/6J/Ibg, DBA/2J/Ibg, BUB/BnJ, ST/bJ) were tested for differences in the propensity to develop conditioned taste aversion. Mice were injected with either nicotine (2 mg/kg) or cyclophosphamide (15 mg/kg) after an ingestion of a novel saccharin solution. Preference testing between water and saccharin was conducted over a 36 day time period. Dose response curves (0-2 mg/kg for nicotine and 0-25 mg/kg for cyclophosphamide) were constructed for 3 of the 6 inbred strains of mice (C3H/2Ibg, C57BL/6J/Ibg, DBA/2J/Ibg). Evidence suggests that there is genetic differences in the acquisition and extinction of conditioned taste aversion and that these differences appear to be dose dependent.

Supported by DA-03194 and DA-00116.

492.18

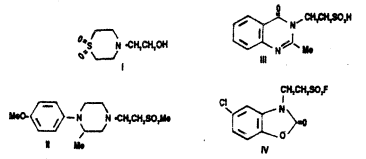
DIFFERENTIAL EFFECTS OF A1 AND A2 ADENOSINE RECEPTOR ANTAGONISTS ON PLACE CONDITIONING AND MOTOR ACTIVITY IN RATS. N.T. Brockwell and R.J. Beninger. Dept. Psychology, Queen's University, Kingston, K7L 3N6, Canada.

The recent development of potent antagonists which are relatively selective for A1 and A2 adenosine receptors has made it possible to assess the role of each receptor subtype in modulating behavior. The present study utilized newly developed apparatus which is capable of simultaneously measuring spontaneous motor activity and place conditioning, the most commonly used behavioral measure of drug reward. The experimental design consisted of 3 phases conducted over 12 successive days. During 3 preconditioning sessions, undrugged male Wistar rats received access to two distinctive chambers connected by a small tunnel. During the 8-session conditioning phase, groups were administered either the A1 antagonist CPX or the A2 antagonist CGS 15943 and confined to one of the chambers. On alternate sessions rats were injected with the vehicle and confined to the opposite chamber. On the test session, undrugged animals were again allowed access to both chambers. Results indicate that both 0.1 and 1.0 mg/kg IP CGS 15943 produced significant hyperactivity; 1.0 mg/kg IP CGS 15943 also produced a significant place preference. CPX (0.1 and 1.0 mg/kg IP) failed to significantly alter activity or induce place conditioning. Using an identical procedure, (+)-amphetamine (2.0 mg/kg IP) produced both significant hyperactivity and a place preference as expected. These results suggest the positive behavioral effects previously found with the non-specific adenosine antagonist caffeine (Brockwell, Eikelboom, & Beninger, Neurosci. Abstr., 1988) may be mediated by the A2 receptor subtype. (Funded by NSERC)

492.19

EFFECT OF SYNTHETIC ANALOGS OF TAURINE ON AUDIOGENIC SEIZURES, ETHANOL-INDUCED SLEEPING TIMES AND SYNAPTOSOMAL CALCIUM BINDING. R.J. Huxtable, R. Gupta*, R. Bowers* and K. Izumi**). Department of Pharmacology, University of Arizona, College of Medicine, Tucson, Arizona 85724; *)Department of Pharmacology, Faculty of Medicine, Kagoshima University, Kagoshima 890, Japan.

Taurine occurs in mM concentration in the brain. In vitro, taurine (25 mM) stimulates the high affinity binding of 6 μM Ca^{2+} to brain synaptosomes in a manner which is antagonized by the taurine antagonist, TAG (*Pharmacologist 30, A86, 1988*). Analogs of various structural types have now been synthesized which also produce a TAG-antagonized stimulation of Ca^{2+} binding. This **I** (10 mM) increases the binding of Ca^{2+} by 18%. Additionally, other compounds inhibit the binding of Ca^{2+} to brain synaptosomes in a manner antagonized by taurine itself. Thus, **II** (25 mM) produces a 34% decrease in Ca^{2+} binding. Taurine significantly reduces ethanol-induced sleeping time in mice without affecting blood ethanol concentration. A number of analogs mimic this action. For example, **III** (0.43 mMol/kg i.p.) significantly reduced sleeping time from 82 min to 66 min. Certain analogs also offered protection against sound-induced seizures in rats. Thus, **IV** (0.3 mMol/kg^{-1} i.p.) prevented seizures in 3 of 6 rats of the minimal seizure strain and 3 of 4 rats of the maximal seizure strain.



CELLULAR BIOLOGY OF 5HT RECEPTORS

493.1

FUNCTIONAL EVALUATION OF SEROTONIN 1C AGONISTS AND ANTAGONISTS BY INTRACELLULAR CALCIUM RELEASE AND PHOSPOINOSITIDE HYDROLYSIS IN A CELL LINE EXPRESSING A RECOMBINANT SEROTONIN 1C RECEPTOR. M. Baez, P. A. Hyslop* J. E. Audia, P. Q. Mooney* and L. Yu+. Lilly Research Laboratories, Lilly Corporate Center, Indianapolis, IN 46285; +Dept. of Medical Genetics, Indiana University School of Medicine, Indianapolis, IN 46223 USA.

AV12 cells were stably transformed with an expression vector, pHD, containing a full length clone of the mouse serotonin (5HT) 1C receptor. RNA hybridization and ligand binding studies confirmed the expression of the 5HT1C receptor in the recombinant cell line A1C-19. Stimulation of the A1C-19 cells with 5HT and known 5HT1C agonists resulted in release of intracellular calcium which was detected by dye fluorescence. This response was blocked by known 5HT1C receptor antagonists. The functional coupling of the 5HT1C receptor to phospholipase C in A1C-19 cells was further demonstrated by increased phosphoinositide hydrolysis in response to 5HT. These studies indicate that the A1C-19 cell line will be useful in evaluating novel 5HT1C receptor agonists and antagonists.

493.2

REGULATION BY GUANINE NUCLEOTIDES OF MEMBRANE BOUND AND SOLUBILIZED MELATONIN RECEPTORS FROM CHICKEN BRAIN. K.C. Chung and M.L. Dubocovich. Dept. Pharmacology, Northwestern Univ. Med. Sch., Chicago, IL 60611.

In this study we examined the effect of guanine nucleotides on the binding of the melatonin agonist 2-[^{125}I]-iodomelatonin (2-IMEL) in P_2 membranes and (0.5 %) digitonin-solubilized fractions of chicken brain (5 weeks old). These fractions were prepared in 50 mM Tris-HCl buffer (20 % glycerol, 1 mM EDTA, 1 mM PMSF, 1 mM mercaptoethanol, 10 mM Mg_2Cl, pH 7.4). The affinity (Ki) of 6-chloromelatonin for 2-IMEL was lower in solubilized fractions before (12 nM) or after (16.6 nM) elution through a G-100 column, than in membrane fractions (Ki = 214 pM). Guanine nucleotides inhibit 2-IMEL binding in membranes [GTPγS (Ki = 900 nM) < GTP << GMP], but not in the soluble fraction. GTPγS (0.1 mM) decreased the Bmax from 14.2 to 6.1 fmol/mg protein with no change in the K_D (234 pM) in membranes, but not in the soluble fraction (K_D = 44.5 ± 17 pM, Bmax = 4.1 ± 1.6 fmol/mg protein, n=3). Following reconstitution of CHAPS solubilized fractions with asolectin the GTPγS sensitivity for 2-IMEL binding was restored. We conclude that decoupling of melatonin receptors from G-proteins may account for the reduced affinity of 2-IMEL in solubilized fractions. Supported by USPHS grants MH 42922 (MLD) and NS 01740 (KCC).

493.3

SEROTONIN POTENTIATES ADENOSINE RECEPTOR-MEDIATED INCREASES OF cAMP IN MICROVASCULAR SMOOTH MUSCLE CELLS IN CULTURE. K.A. Berg[1], C.A. Diglio[2] and S. Maayani[1,3]. Depts. of Anesthesiology[1] and Pharmacology[3], Mount Sinai School of Medicine, CUNY, NY, NY 10029, and Dept. of Pathology[2], Wayne State University School of Medicine, Detroit, MI 48201.

Cerebral blood flow is modulated by alteration of blood vessel tone. This alteration can be achieved by simultaneous activation of multiple receptor-linked signal transduction pathways. We have chosen, as a model system, to study the effect of 5-hydroxytryptamine (5-HT, serotonin) on adenosine receptor-mediated cAMP production in vascular smooth muscle cells derived from rat cerebrocortical resistance vessels. Incubation (15 minutes) with 5'-N-ethylcarboxamidoadenosine (NECA, adenosine A_2 agonist; EC_{50}= 2 μM) increased cAMP production a maximum of 4-5 fold. 5-HT (10 μM) alone produced only a small increase in cAMP levels, however, it increased (2-fold) the maximal cAMP produced by NECA and shifted the NECA concentration response curve to the left (2-3 fold). The EC_{50} for 5-HT was approximately 1 μM. In addition, forskolin (1 μM)-stimulated cAMP levels were also enhanced by 5-HT. The potentiation of NECA-induced cAMP production by 5-HT was antagonized by the presence of the 5-HT_2 antagonists, ketanserin and spiperone, and mimicked by the selective 5-HT_2 agonist, 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI). Incubation with the active phorbol ester, phorbol 12-myristate 13-acetate (PMA, 1 μM), but not the inactive phorbol ester, 4α-12,13-didecanoate (PDD, 1 μM), enhanced the cAMP response to NECA in a fashion comparable to that of 5-HT. Furthermore, protein kinase C (PKC) activity was increased (approximately 60%) following incubation with 5-HT for 15 minutes. These data suggest that the potentiation of NECA- and forskolin-induced cAMP production in resistance vessel smooth muscle cells by 5-HT is mediated via 5-HT_2 receptor activation of PKC. (USPHS GM-34852, AHA Grant in Aid 890750)

493.4

5HT RECEPTORS ON CARCINOID TUMOR CELLS IN CULTURE
Haber, B., Lakoski*, J., Black*, E., Karp*, G., Parekh*, D., Townsend*, C., Thompson*, J. C., and Ishizuka*, J. Marine Biomed. Inst., Depts. of Pharmacol., Surgery, and Human Biol. Chem. and Genet., Univ. of Texas Med. Branch, Galveston, TX 77550

Cultured BON cells of carcinoid tumor origin contain high basal levels of 5HTP, 5HT, and 5HIAA as well as chromogranin, calcitonin, neurotensin, and pancreastatin (PS). 5HT and PS are released from BON cells by Ach (blocked by atropine) and by isoproterenol. 10^{-8} M 5HT. BON cells release PS in vitro upon stimulation with The presence of binding sites for a 5HT autoreceptor on cultured BON cells was assessed using [^{3}H]8-OH-DPAT (8-hydroxy-2(di-n-propylamino)tetralin selective agonist for $5HT_{1A}$ binding site. Scatchard analysis was done on a P2 tissue suspension prepared in Tris buffer (pH 7.4). Data shows significant numbers of $5HT_{1A}$ binding sites on BON cells (B_{max} +41.7+13.7 fmol/mg prot.) with a K_d of 3.0+1.8nM. Additional controls included analysis of hippocampal tissue with expected K_d(9.1nM) and B_{max}(445 fmol/mg prot.). Taken together, these data suggest that the release of 5HT from BON cells in vitro and carcinoid tumors in vivo may regulate the release of PS and other peptides via $5HT_{1A}$ autoreceptors.

Supported by ACS PDT-220, a grant from Sigma Tau and AG00450.

493.5

DOWN-REGULATION OF THE $5HT_{1B}$ RECEPTOR IN OPOSSUM KIDNEY (OK) CELL MEMBRANES R.C. Pleus and D.B. Bylund, Dept of Pharmacol, Univ of Neb Med Ctr, Omaha, NE, 68198.

We are investigating the regulation of the $5HT_{1B}$ receptor using membrane binding studies and functional assays. We observe a 40% decrease in $5HT_{1B}$ receptors in OK cell membranes when cells are pretreated for 16 hr with 10 μM 5HT and in the absence of fetal bovine serum (-FBS) using ^{125}I-cyanopindolol (1 to 124 pM) as the radioligand. OK cells were plated at $6x10^6$ cells per 150 mm culture dish and grown for 3 or 7 days. Cells harvested on day 7 yielded higher B_{max} values (~ 2x). FBS has an effect in these experiments. When cells are exposed to 5HT in the presence (+) of FBS, B_{max} values are not different, however, control -FBS have higher B_{max} values than +FBS.

		Day Harvested	K_D (pM)	B_{max} fmol/mg protein	n
Control	- FBS	7	48 ± 4	71 ± 14	3
	+ FBS	7	54 ± 4	49 ± 6	2
	+ FBS	3	40 ± 3	26 ± 2	4
5HT	- FBS	7	68 ± 12	43 ± 10	3
	+ FBS	7	76 ± 29	34 ± 8	2
	+ FBS	3	40 ± 12	22 ± 3	4

(*control -FBS vs 5HT -FBS, B_{max} p = 0.03, K_D p = 0.1115; control +FBS vs 5HT +FBS, B_{max} p = 0.095, K_D p = 0.479, one-tailed paired t-test)

In a functional assay (5HT inhibition of parathyroid hormone (PTH) stimulated cAMP) using intact OK cell cultures under -FBS conditions (as above), 5HT inhibits PTH-stimulated cAMP in a dose-response manner. When 0.1 to 10% of FBS is added to the assay, FBS in the presence of PTH inhibits cAMP production in a dose-dependent manner and is attenuated by 2 μM methiothepin. These studies demonstrate 1) down-regulation of the $5HT_{1B}$ receptor and 2) an FBS-related effect, which should be considered when undertaking such studies. (NIH grant GM37664)

493.6

REGULATION OF 5-HT_2 RECEPTORS ON P11 CELLS BY SEROTONIN. K.J. Ivins and P.B. Molinoff. Department of Pharmacology, University of Pennsylvania, Philadelphia, PA 19104.

P11 cells express a high density of 5-HT_2 receptors coupled to phosphoinositide (PI) hydrolysis. The effect of exposure to serotonin (5-HT) on the expression and function of 5-HT_2 receptors in P11 cells was investigated. In these experiments, PI hydrolysis was measured by the accumulation of ^{3}H-inositol phosphates in the presence of LiCl and drugs. The ability of 5-HT to stimulate PI hydrolysis was greatly reduced or absent following exposure of P11 cells to 10 μM 5-HT for 8 hr. The desensitization of 5-HT_2 mediated PI hydrolysis by 5-HT was time- and concentration-dependent. Exposure of P11 cells to 10 μM 5-HT for as short a time as 20 min led to a decrease in the ability of 5-HT to stimulate PI hydrolysis. With longer periods of exposure, concentrations of 5-HT as low as 100 nM caused desensitization of PI hydrolysis. Stimulation of PI hydrolysis by the α_1-adrenergic agonist 6-fluoronorepinephrine was not affected by prior exposure of cells to 5-HT, suggesting that the desensitization was selective for 5-HT_2 mediated PI hydrolysis. The density of 5-HT_2 receptors in membranes prepared from P11 cells was measured using ^{125}I-LSD. Exposure of P11 cells to 5-HT resulted in a decrease in the density of 5-HT_2 receptors with no change in the affinity of the receptors for ^{125}I-LSD. The decrease in the density of receptors was rapid and pronounced. A decrease in receptor density was observed following exposure to 10 μM 5-HT for 1 hr; after 24 hrs the density of 5-HT_2 receptors was approximately 20% of the original value. A decrease in the density of 5-HT_2 receptors after exposure of P11 cells to 5-HT may be involved in desensitization of 5-HT_2 mediated PI hydrolysis. (Supported by USPHS NS18591).

493.7

GENERATION OF A DNA PROBE FOR THE $5HT_{1b}$ RECEPTOR IN THE OK CELL, AN OPOSSUM KIDNEY CELL LINE. D.R. Cerutis and D.B. Bylund. Department of Pharmacology, University of Nebraska Medical Center, Omaha, NE 68198-6260.

Previous studies from our laboratory (Murphy, T.J., and Bylund, D.B., Mol. Pharmacol. 34:1-7, 1988) have demonstrated the presence of the serotonin $5HT_{1b}$ receptor in the OK cell line, an established renal proximal tubule epithelial cell line. In order to generate a probe for this receptor, the published sequences of G-21, $5HT_{1c}$ and 5HT-2 were used to design degenerate primers directed at the highly conserved sequences in the third and sixth transmembrane domains. OK cell RNA was isolated using an Applied Biosystems nucleic acid extractor. Approximately 2μg of this RNA was reverse transcribed using Moloney Murine Leukemia Virus reverse transcriptase and the resulting cDNAs subjected to 30 cycles of PCR with the above primers. The resulting product of about 0.7 kb is consistent with the calculated size of this region from the published sequences of other serotonin receptors. This 0.7 kb fragment also hybridizes with an oligonucleotide directed towards the fifth transmembrane region of the other published serotonin receptors. This 0.7 kb product is currently being used to screen a lambda gt11 OK cell cDNA library. (supported by NIH grant GM 40784)

493.8

PHARMACOLOGICAL CHARACTERIZATION OF A CLONED HUMAN 5-HT_{1D} RECEPTOR. J. M. Zgombick, R.L. Weinshank', M. Macchi', P. Hartig, and T.A. Branchek. Neurogenetic Corporation, Paramus N.J. 07652

We have recently isolated a DNA sequence encoding a human 5-HT_{1D} receptor. This new serotonergic receptor gene encodes a protein whose amino acid sequence displays relatively low transmembrane homology to other human G protein-coupled receptors: 5-HT_{1A} (56%), 5-HT_2 (43%), dopamine D_2 (46%), and α_{2A} (45%). Murine fibroblast Ltk' cells were stably transfected with the DNA encoding this newly derived human receptor gene, membranes were harvested, and radioligand studies were conducted. Occupancy studies demonstrated that [^{3}H]-5HT bound with high affinity (K_d=3.9±0.9 nM) and in a saturable manner (B_{max}=4.4±1.0 pmol/mg prot.). Competition studies revealed a pharmacological profile with rank order of potency: 5CT ≥ 5HT >> TFMPP > DPAT > DOI > Pindolol > ICS 205-930. The coupling of this receptor to G protein(s) was assessed in binding studies using the non-hydrolyzable guanine nucleotide analog, 5'-guanylylimidodiphosphate (Gpp(NH)p). Gpp(NH)p decreased specific [^{3}H]-5HT binding by 25±3 % in a concentration-dependent manner with an IC_{50} of 4.1±1.1 μM. These properties are consistent with the identification of this new clone as a human 5-HT_{1D} receptor. The existence of multiple subtypes of 5-HT_{1D} receptor has been suggested previously (Xiong and Nelson, Life Sci. 45:1433, 1989). The present clone will provide a useful tool to explore this possibility in the human genome. Further, it has been shown that closely related receptors of the same subfamily (e.g. α_{2A}, α_{2B}, α_{2C}) generally display approximately 75% transmembrane homology; in contrast, receptors from different subfamilies (e.g. 5-HT_{1A} vs. 5-HT_2) exhibit only 45-50% transmembrane homology. Therefore, we propose that the cloned human 5-HT_{1D} receptor constitutes the first member of a new subfamily of serotonin receptors.

493.9

DENSITY-DEPENDENT REGULATION OF AFFINITY STATES OF THE CLONED HUMAN 5-HT_2 RECEPTOR. N. Adham*, M. Macchi*, H-T. Kao*, P. Hartig, and T. Branchek. Neurogenetic Corporation, Paramus, N.J. 07652

We have recently reported that the cloned human 5-HT_2 receptor binds both ^{3}H-DOB and ^{3}H-ketanserin, supporting the hypothesis that the DOB binding site represents the agonist high affinity state of the 5-HT_2 receptor. (Hartig et al, ACNP 1989; Branchek et al, 2nd IUPHAR Serotonin Satellite, 1990). However, we noted that the data obtained in our laboratory displayed a markedly higher proportion of high affinity sites than reported by others and that this ratio could be experimentally manipulated. In order to address possible factors regulating the expression of multiple affinity states of the 5-HT_2 receptor, we made use of the high specific activity radioligand ^{125}I-DOI (Dupont-NEN). Specific binding of ^{125}I-DOI to the stable transfectants (Ltk$^-$) displayed high affinity (K_d=0.22nM) and saturability (B_{max}=124 fmol/mg prot). The density of ^{125}I-DOI binding sites in these cells was approximately half of the total ^{3}H-ketanserin binding sites (B_{max}= 234 fmoles/mg prot). The GTP derivatives GTP-γ-S and GppNHp potently inhibited the binding of ^{125}I-DOI (K_i= 39nM; 256nM, respectively) and produced an effect comparable to that observed in our control rat cortical membranes (K_i= 183 nM; GppNHp). Approximately 50% of the ^{125}I-DOI binding sites were sensitive to guanyl nucleotides. In order to determine the effect of receptor density on the proportion of receptors in the high and low affinity states, we used increasing amounts of DNA encoding the 5-HT_2 receptor in transient transfections (Cos 7). As the DNA was increased (in 5 steps, from 1-10 µg DNA/10^6 cells), we detected an increase in both the ^{125}I-DOI and ^{3}H-ketanserin binding; however, the ratio of ^{125}I-DOI/^{3}H-ketanserin bound (fmol/mg protein) decreased from 1.8-0.15. Furthermore, we noted a similar decrease in the proportion of high affinity states as a function of confluence. Although the number of receptors in the high affinity state did not change, the density of ^{3}H-ketanserin binding sites increased dramatically as confluence increased, thus decreasing the ratio of high/low affinity states. These data indicate that the pool of endogenous G-protein(s) may be rate-limiting in these expression systems such that the proportion of receptors in the high affinity state (presumably coupled to G-proteins) decreases as the receptor density increases.

493.10

BENZAZEPINE FUNCTION AT 5-HT_{1c} RECEPTORS EXPRESSED IN XENOPUS OOCYTES. C.A. Briggs, N.J. Pollock, D.E. Frail, R.F. Rakowski*, and J.W. Kebabian. Depts. Neuroscience & Corp. Molecular Biology, Abbott Labs, and Dept. Physiology, Chicago Medical School, North Chicago, IL.

Xenopus laevis oocytes were prepared and injected using standard techniques; two-electrode voltage clamp was used to record the Ca^{2+}-dependent Cl^- current response to 5-HT and other agonists. After injection with **rat brain RNA**, oocytes responded to 5-HT with an EC_{50} of about 30 nM, consistent with a selective expression of 5-HT_{1c} receptors (Lubbert et al. (1987), *J. Neurosci.* 7:1159). SCH 23390 (10^{-7}-10^{-5} M) blocked the response to 5-HT (10^{-7} M) in a stereo-selective and dose-dependent manner. There was no apparent agonist activity with SCH 23390. After injection with **clonal 5-HT_{1c} RNA**, oocytes responded to 5-HT with an EC_{50} of 10-30 nM. SCH 23390 (10^{-8}-10^{-4} M) was a stereo-selective partial agonist (EC_{50} about 4 uM, *cf.* Hoyer et al.(1989) *N.-S. Arch. Pharmac.* 339: 252). SKF 38393 and dopamine (10^{-6}-10^{-4} M) also acted as agonists. Benzazepines selective for the D1 dopamine receptor also bind and function at the 5-HT_{1c} receptor. Oocytes can be used to study such functions with normal and mutagenized RNAs.

493.11

MAMMALIAN CELLS TRANSFECTED WITH A RAT 5HT_2 RECEPTOR cDNA: EVIDENCE FOR MULTIPLE STATES AND NOT MULTIPLE 5HT2 RECEPTOR SUBTYPES. S. Leonhardt, E. Weisberg, B.J. Hoffman*, and M. Teitler. Dept. of Pharmacology and Toxicology, Albany Medical College, Albany, N.Y., 12208; *Lab. of Cell Biology, NIMH, Bethesda, Maryland 20892

Evidence has accumulated that the 5HT_2 receptor exists in two different states. The agonist high affinity state is coupled to a G-protein and can be labelled with the radioactive hallucinogens ^{3}H-DOB, ^{125}I-DOI and the antagonist ^{3}H-Ketanserin. The agonist low affinity state consists of the free receptor and is labelled by ^{3}H-Ketanserin. Recently, an alternative hypothesis has been put forward proposing that the radioactive hallucinogens are labelling a 5HT_2 receptor sub-type distinct from the receptor labelled by ^{3}H-ketanserin and other antagonist radioligands. To show which of these hyoptheses is correct, the rat 5HT_2 receptor gene was transfected into NIH-3T3 (murine fibroblast) and COS (green monkey kidney) cells. Neither non-transfected cell line expresses the 5HT_2 receptor. The transfected cells expressed high affinity binding sites for both ^{125}I-DOI and ^{3}H-ketanserin. Agonist affinities were significantly higher for ^{125}I-DOI labelled receptors than for ^{3}H-ketanserin labelled receptors in both cell types. Under the same experimental conditions the affinity of agonists and antagonists for the binding sites were essentially identical to the affinities of these sites in mammalian brain homogenates. The ^{125}I-DOI binding was guanyl-nucleotide sensitive, indicating coupling to a GTP- binding protein. These data indicate that the 5HT_2 receptor gene encodes for the agonist high affinity state and the agonist low affinity state of the 5HT_2 receptor. These results strongly support the two state receptor hypothesis and do not support the multiple 5HT_2 receptor sub-type hypothesis.

493.12

5-HT1A RECEPTORS REGULATE ADENYLATE CYCLASE MEDIATED RESPONSES IN THE HIPPOCAMPUS. Rodrigo Andrade, Dept. of Pharmacology, St. Louis Univ. School of Med. St. Louis, MO 63104

Although electrophysiological studies have shown that 5-HT1A receptors in the CA1 region of the hippocampus elicit a hyperpolarization mediated by an increase in potassium conductance through a mechanism that is independent of cAMP, biochemical experiments have reported that these receptors can also regulate adenylate cyclase. These results suggested that 5-HT1A receptors might be able to regulate responses signalled through cAMP generation. To test this possibility I examined the effects of the 5-HT1 agonist 5-carboxyamidotryptamine (5-CT) on β-adrenergic responses using intracellular recordings in *in vitro* hippocampal slices.

Consistent with previous reports, the predominant effect of norepinephrine (NE) in this region was to reduce the calcium activated afterhyperpolarization (AHP) by acting on a β-adrenergic receptor (Madison and Nicoll, 1982). Bath administration of 5-CT (0.3µM) elicited a hyperpolarization and a decrease in the amplitude of the AHP which appeared by in large secondary to the increase in membrane conduce. Moreover, in the presence of 5-CT, NE was proportionally more effective in reducing the AHP than either under control conditions or following the removal of the 5-CT from the bath (mean enhancement of NE: 27%, range 16%-49%, $p<0.01$). Similar effects were also observed in response to baclofen, a GABA-B agonist that has been previously shown to also inhibit adenylate cyclase and to converge upon a the same transmembrane signalling mechanism as 5-HT1A receptors to open potassium channels in these cells (mean enhancement= 23%, range 8%-40%, $p<0.05$).

These results indicate that activation of 5-HT1A and GABA-B receptors fail to block responses signalled by activation of adenylate cyclase and indeed results in an enhancement of these responses. These results are consistent with the biochemical observation that at least some receptors coupled to Gi can enhance the effects of β-adrenergic stimulation of adenylate cyclase and suggest a possible physiological role for such an enhancement. Supported by grant MH 43985 and the Alfred P. Sloan Foundation

NEURAL-IMMUNE INTERACTIONS: STRESS AND BEHAVIOR

494.1

MECHANISMS OF SUPPRESSED ANTIBODY PRODUCTION IN HANDLED MICE. J.M. Dopp, S.G. Schmidt*, N. Cohen*, and J.A. Moynihan*. Depts. of Microbiology and Immunology, and of Psychiatry, University of Rochester School of Medicine and Dentistry, Rochester, NY, 14642.

Handled mice show behavioral and physiological responses indicative of arousal, with presumed neuroendocrine and sympathetic nervous system concomitants. Thus, handling has been used as a rodent model of human stress. Mice handled before immunization show decreased primary IgG antibody levels and mitogen-induced T cell proliferation, but unchanged numbers of total spleen cells and percentages of splenic B cells, T cells, and T cell subsets (Moynihan, et al., Life Sci., in press). To determine the mechanism(s) through which handling suppresses antibody production, we have analyzed some of the known components of the immune response to keyhole limpet hemocyanin (KLH).

Group-housed female BALB/c mice were held individually 2 min/day for 2 weeks, while controls remained in their cages. 24 hr later, mice were either sacrificed (Group 1) or injected i.p. with 100 µg KLH (Group 2) and sacrificed 3 or 6 days later Compared to controls, handled mice in Group 1 had fewer Ig-secreting B cells (CELISA) and decreased levels of spontaneously-produced IgM (ELISA), but unchanged T-cell secretion of Con A-induced interleukin-2 (IL-2) and/or IL-4 (CTLL assay). Handled mice in Group 2 showed diminished KLH-induced proliferation (^{3}H-TdR incorporation) of unfractionated spleen and mesenteric lymph node cells.

The results indicate that neuroendocrine and/or sympathetic responses which accompany handling can alter specific immune function as measured by antibody production. Dimished antibody production does not appear to be caused by a decrease in IL-2/4, known to play a role in B cell differentiation. Future studies will focus on levels of T-cell help and on other relevant lymphokines and CNS-derived products that might directly affect immune responses.

494.2

SURGICAL STRESS INCREASES THE NUMBER OF BREAST CANCER METASTASES IN RATS G.G. Page, S. Ben-Eliyahu, R. Yirmiya, & J.C. Liebeskind Dept. of Psychology, UCLA, Los Angeles, CA 90024.

The acute stress of surgery is known to suppress natural killer (NK) cell cytotoxicity and increase the metastasis of solid tumors. Using a tumor model for breast cancer in rats, MADB106 adenocarcinoma, we recently demonstrated that forced swim stress increases metastases by suppressing NK activity against this tumor. This same malignant cell line, MADB106, syngeneic to Fischer 344 rats was used for this study. Thirty-eight rats were divided into 3 groups, subjected to a standard abdominal surgery under halothane anesthesia, to anesthesia only, or to no treatment. Three to five hours after completion of surgery/anesthesia, all animals were injected with $3x10^5$ MADB106 cells in .5ml PBS. Twenty-one days later, lungs were taken and surface metastases counted. Surgical stress significantly increased metastatic growth in the surgery group compared to either the anesthesia only or control groups. Taken together with the established role of NK cells in controlling MADB106 metastases, and with our recent evidence for a causal relationship between the effect of swim stress on NK activity and on metastases, the present finding suggests that surgical stress increases metastases by suppressing NK activity. This hypothesis is now being tested directly in our laboratory. Supported by the UCLA Psychoneuroimmunology Program (S.B-E. & R.Y.), and NIH grant NS 07628.

494.3

OPIATE-INDUCED INCREASE IN TUMOR METASTASES IS CAUSED BY SUPPRESSION OF NK ACTIVITY AND IS NOT MEDIATED BY GLUCOCORTICOIDS. R. Yirmiya, S. Ben-Eliyahu, Y. Shavit, R. P. Gale, A. N. Taylor, H. Weiner, G. Page, N. Lee*, and J. C. Liebeskind. University of California, Los Angeles, CA 90024, and the Hebrew University of Jerusalem, Israel.

The effects of two opiate drugs, morphine and fentanyl, on metastatic tumor growth, were tested using the MADB106 mammary adenocarcinoma cell line, previously shown to be controlled by natural killer (NK) cells. Additionally, the cytotoxic activity of NK cells against this tumor, which is syngeneic to Fischer 344 rats, was measured *in vitro*. Fischer 344 rats were injected either with naltrexone (10 mg/kg) or saline, and 20 min later, were injected with either morphine (50 mg/kg), fentanyl (35 µg/kg) or saline. One hour after the second injection, animals were either injected intravenously with 10^5 MADB106 cells and two weeks later lungs were removed and surface metastases counted, or sacrificed and NK activity determined in a standard ^{51}chromium-release assay. Administration of morphine or fentanyl produced a 5-8 fold increase in number of MADB106 metastases *in vivo*, and markedly decreased NK cytotoxicity *in vitro*. Naltrexone completely blocked these effects. In another experiment we found that administration of the glucocorticoid antagonist RU38486 (25 mg/kg, one hour before morphine administration) did not attenuate morphine-induced increase in lung metastases, indicating that the effects of morphine are not mediated by glucocorticoids. These findings suggest a casual relationship between the immune suppressive and tumor enhancing effects of opiates. Supported by the UCLA Psychoneuroimmunology Program (R.Y. and S.B.-E.), and grants NS 07628 (J.C.L.), AA 06744 and VA Medical Research Service (A.N.T).

494.4

SYMPATHETIC INVOLVEMENT IN THE STRESS-INDUCED INCREASE OF METASTATIC SPREAD: STUDIES OF A NATURAL KILLER-SENSITIVE TUMOR S. Ben-Eliyahu, R. Yirmiya, G. Page, S. A. Boun, A. N. Taylor, R. P. Gale, H. Weiner, and J. C. Liebeskind Dept. of Psychology, UCLA, Los Angeles, CA 90024

Stress can suppress natural killer (NK) cell activity and increase tumor growth. We have recently provided evidence for a causal relationship between immunosuppressive and tumorigenic effects of stress in an animal model of breast cancer. Using the MADB106 tumor cell line, which is syngeneic to the Fischer 344 rats used in these studies and is known to be controlled by NK cells, we previously showed that stress suppressed NK activity against this tumor in vitro and increased the number of metastases in rats inoculated with this tumor. In the present study, rats were either subjected to intermittent forced swimming stress or were not stressed. In the first experiment each group was injected with the ganglionic blocker, chlorisondamine (3 mg/kg i.p.), or with saline. In the second experiment each group was either injected with the selective ß-2 adrenergic antagonist, butoxamine (25 mg/kg i.p.), or with saline. One hour after stress rats were injected i.v. with $2x10^5$ MADB106 cells, and 12 days later surface lung metastases were counted. Stress significantly increased number of metastases in both experiments; clorisondamine markedly decreased this elevation, and butoxamine completely blocked it. Neither drug affected number of metastases in nonstressed groups. These findings suggest that ß-2 adrenergic receptor activation by the sympathetic nervous system is important in mediating the effects of stress on metastatic growth. Supported by the UCLA Psychoneuroimmunology Program (S. B.- E. and R.Y.) and grants NS 07628 (J.C.L.), AA 06744 and VA Medical Research Service (A.N.T)

494.5

EFFECTS OF EXERCISE ON IMMUNE FUNCTIONS OF UNDERNOURISHED MICE. S. Filteau*†, M.P. O'Grady†, R.A. Menzies*†, T. Kaido*†, J.B. Gelderd^, C. Edwards≠, and N.R.S. Hall†. Dept. of Physiology≠, and Dept. of Psychiatry†, Univ. of South Florida College of Med., Tampa, FL 33613, and Dept. of Anatomy^, Texas A&M Univ., College Station, TX 77843-1114.

Anorexia nervosa patients generally have normal immune function and viral disease resistance in spite of their severe undernutrition. Possibly the intense exercise commonly performed by these patients helps prevent undernutrition-induced immunodepression. To test this hypothesis, mice were fed either *ad libitum* or in restricted quantities to induce 25% loss of initial weight over 3 weeks. Half the animals from each dietary group were run on a treadmill for 30 min/day, 5 days/week. Exercise had no effect on several measures of nutritional status. One third of the restricted non-exercised mice, but no mice from any other group, exhibited intestinal pathology. Spleen weight and blastogenic response to lipopolysaccharide were significantly increased by exercise in undernourished mice. *In vivo* antibody response to sheep red blood cells, *in vitro* splenic responses to Concanavalin A and phytohemagglutinin, and production of tumor necrosis factor were not significantly affected by exercise. Serum corticosterone level was increased by food restriction and significantly decreased by exercise in the undernourished mice. These values are being correlated with changes in brain levels of biogenic amines. Regular exercise may help prevent undernutrition-induced immunodepression by altering the response of animals to stress. Supported in part by NIMH (MH45646) and the Med. Res. Council of Canada.

494.6

THE EFFECTS OF ISOLATION-INDUCED AGGRESSION ON IMMUNE PARAMETERS IN MICE. M.P. O'Grady and N.R. Hall. Div. of Psychoimmunology, Dept. of Psychiatry, Univ. of South Florida College of Medicine, Tampa, FL 33613

Isolation-induced aggression (IIA) was used as a model to assess the effects of a behavioral manipulation on measures of immunity. In addition, we evaluated the ability of thymosin fraction 5 (TF5), a thymic extract, to modulate IIA. TF5 stimulates ACTH release which, in turn, has been found to reduce IIA.

Pretreatment with TF5 altered neither the number of fights nor the latency to attack. However, the fighters and the intruders differed in the response of their lymphocytes to mitogens. Compared to intruders, the fighters were suppressed in their response to Con A ($p<.02$) and Pokeweed ($p=.001$) with no change in response to PHA. We then investigated the effect of housing and intensity of fighting on thymus weight. The intensity of the fights was determined by the housing conditions of the participants. Animals who fought submissive intruders as well as those who fought other aggressive mice had reduced thymus weights compared to the isolated controls ($p<.04$). Interestingly, there was a difference between the two groups of fighters as a function of intensity of fights. The group who fought aggressive mice had smaller thymuses than those animals who fought submissive intruders ($p=.006$). Thus, thymus weights reflected these intensity differences in what could be construed as a behavioral "dose-response curve". Research was supported in part by NS 21210.

494.7

BRAIN C-FOS IMMUNOREACTIVITY INDUCED BY CONDITIONED AND UNCONDITIONED AVERSIVE STIMULI.
M.O. Fraser*, G.E. Hoffman, D.T. Lysle, J.E. Cunnick, M.A. Pezzone, B.J. Kucinski and B.S. Rabin Departments of Pathology and Physiology, University of Pittsburgh, Pittsburgh, PA, 15216.

A variety of stressors can decrease immune function, and the hypothalamus is implicated in this response. In an attempt to define areas of the brain that influence immune function and respond to stressors, c-fos induction was examined following two stress paradigms. Adult male Lewis rats (2-4 per group) either underwent conditioning to foot shock (unconditioned stimulus, US) using auditory clicks as the conditioned stimulus (CS) or were exposed to the CS alone, and 12 days following the conditioning trials were either exposed to the CS for 1 hour or were taken directly from their cages. Other animals were either exposed to the US alone or remained naive. Animals were prepared for c-fos staining, as a marker for stimulated activity, as previously described (Hoffman et al., Endocrinology 126: 1736, 1990). C-fos was strongly expressed in cells of the paraventricular nuclei, some of which contain CRF, and other hypothalamic areas directly associated with autonomic function, the septal nuclei, and the corticomedial amygdala in animals exposed to a conditioned or unconditioned stress. Midline thalamic, amygdaloid and basal ganglia activation were greatest in the animals exposed to US alone. Control animals exhibited very little c-fos in the diencephalon. C-fos containing brain nuclei common to both stressors can now be targeted for further study aimed at elucidating their role in stress induced immune suppression.

494.8

STRESSOR-INDUCED MODULATION OF IMMUNE FUNCTION: EVIDENCE FOR A CONTEXT DEPENDENT HABITUATION PROCESS.
Donald T. Lysle, Ph.D., Dept. of Psychology, University of North Carolina, at Chapel Hill and Joan E. Cunnick, Ph.D., Dept. of Pathology, University of Pittsburgh.

Our prior work using male Lewis rats has shown that presentation of a stressor induces suppression of the mitogenic responsiveness of splenic lymphocytes, and repeated exposure to the stressor will result in attenuation of the suppressive effect. The present study evaluated whether presentations of a stressor (administration of 5.0 mg/kg of Amphetamine) would attenuate the suppressive effect induced by a different stressor (16 presentations of a 5-sec, 1.6 mA electric foot-shock). The results showed that the attenuation or habituation effect was specific to the repeatedly presented stressor, and did not attenuate the suppressive effect of a different stressor. Moreover, subsequent experiments showed that the habituation effect was specific to the environment or context in which the stressor was presented, as presentation of the same stressor in a different context resulted in a renewal of the suppressive property of the stressor. These findings indicate that the habituation process is dependent upon the presence of conditioned stimuli that predict the occurrence of the stressor. Our interpretation suggests that conditioned stimuli produce a compensatory response that opposes the suppressive effect of a stressor.

494.9

STRESS-INDUCED SUPPRESSION OF THE CELLULAR IMMUNE RESPONSE TO HERPES SIMPLEX VIRUS (HSV) AND THE EFFECT ON THE ESTABLISHMENT OF ACUTE AND LATENT INFECTION. R. H. Bonneau*, J. F. Sheridan*, N. Feng*, F. L. Jordan and R. Glaser*. Department of Medical Microbiology and Immunology, College of Medicine; Section of Oral Biology, College of Dentistry. The Ohio State University, Columbus, OH 43210.

It has been well documented that acute or repeated stressful events are able to suppress a broad spectrum of both humoral and cellular immunological responses; however, the effect of stress on the development of specific antiviral immune responses has yet to be reported. We have utilized an established murine model of local and systemic Herpes simplex virus type 1 (HSV-1) infection to study the effect of restraint stress on the generation of HSV-specific cytotoxic and helper T lymphocytes (CTL and HTL) as well as natural killer (NK) cell activity. These studies were extended to examine the effect of stress on the development and restimulation of the HSV-specific memory component of the cellular immune response. In addition, the effect of stress on the establishment of acute and latent HSV infection was investigated. Overall, restraint stress was shown to depress the generation of HSV-specific CTL and NK cell activity following local infection as well as increasing the level of infectious HSV at the site of local infection. Restraint stress was also demonstrated to inhibit the ability to restimulate HSV-specific memory CTL to the lytic phenotype following antigenic challenge. These findings provide evidence that physiological changes associated with restraint stress can influence the ability to generate an immune response to a specific viral infection, thus contributing to the understanding of some of the basic mechanisms underlying stress-associated immune regulation.

494.10

BEHAVIORAL, NEUROENDOCRINE AND IMMUNE SYSTEM STUDIES OF HIGH- AND LOW-AVOIDANCE ROMAN RAT LINES. P. Mormède*, N. Castanon*, *C. Sandi**, S. Vitiello*, J. Dulluc*, M. Le Moal and P.J. Neveu* Lab. Psychobiologie Comportements Adaptatifs INSERM. U259-INRA, Univ. Bordeaux II. Domaine de Carreire 33077 Bordeaux Cedex-France. *Psychobiology Lab., Istituto Cajal, Madrid, Espana.*

Roman strains of Wistar rats have been selected on the basis of their performance in two-way active avoidance behavior and differ also in several other behavioral responses, such as their locomotor activity in a novel environment, the high-avoidance strain (RHA) being more active than the low-avoidance animals (RLA). Despite these marked behavioral differences we could not find any between-strain variation in basal levels of corticosterone and ACTH, in their responses to different protocols of novel environment stress, or after CRF challenge. These results suggest that the differences previously described are not necessarily related to the behavioral trait and may be the consequence of a genetic drift or of local environmental conditions. On the other hand the prolactin response to stress was higher in the low-avoidance line.

The reactivity of spleen lymphocytes was studied *in vitro*. Natural killer activity against YAC-1 tumoral cells as well as the mitogenic response to concanavalin A were much higher in the low-avoidance strain.

The genetic link of neuroendocrine variation and immune function differences with behavioral characteristics is currently under study. The available data suggest that the Roman lines of rats are an interesting model for psychoneuroimmune studies.

494.11

INNATE AND ADAPTIVE IMMUNE RESPONSES IN A SOCIAL CONFLICT PARADIGM M. Lyte[1], S. Nelson[1]* and M.L. Thompson[2]. [1]Biological Sciences, Mankato State University, Mankato, MN 56002 and [2]Biochemistry, Tufts Univ. Sch. of Medicine, Boston, MA 02111.

Innate and adaptive immunity was examined at an *in vitro* and *in vivo* level in three strains of mice subjected to social conflict. C57BL/6, DBA/2 and B6AF1 strains were selected on the basis of known differences in neuroendocrine response to social conflict stress. Generation of primary IgM antibody responses to the T dependent antigen KLH was suppressed following chronic (>1 day) stress periods while IgM response to the T-independent antigen PVP was not affected. *In vitro* proliferative responses of splenocytes to the T cell mitogen Con A and B cell mitogen LPS were unaffected. Acute stress dramatically increased splenocyte phagocytosis while mitogen responses remained unaffected. DBA averaged a 269% increase in phagocytosis as compared to a 412% increase in C57. These findings indicate that alterations in innate immunity in addition to adaptive immunity should also be considered when evaluating neuroendocrine and immune interactions in response to stress and emphasize the importance of using a biologically relevant stressor such as social conflict.

494.12

IMMUNOSUPPRESSION INDUCED BY A MILD STRESSOR OR STRESSOR-RELATED ODORS. S. Zalcman, L. Kerr* and H. Anisman. Dept. of Psychology, Carleton University, Ottawa, Ontario K1S 5B6, Canada.

Paralleling the effects of inescapable footshock, exposure to a mild stressor (165 presentations of a weak light stimulus over a 96 min period) applied 72 hr after sheep red blood cell (SRBC) inoculation resulted in a marked suppression of the plaque forming cell (PFC) response in CD-1 mice. This treatment also reduced antibody titers, but over the course of several experiments this outcome occurred less reliably. A more pronounced and reliable immunosuppression was noted when the stressor was presented to mice in their home cages. Finally, exposing mice to an environment containing odors of mice that had previously been stressed likewise resulted in an immunosuppression. No such effect was evident if mice were exposed to odors of nonstressed mice. It seems that the PFC and antibody responses are exquisitely sensitive to apparently mild stressors, and raises the possibility that a light stimulus might be inappropriate as a CS for certain conditioned immunological studies.

494.13

NEONATAL HANDLING AFFECTS IMMUNE FUNCTION AND BEHAVIOR IN PORSOLT'S SWIM TEST. E. Fride, L.A. Hilakivi, and P.K. Arora*. Lab. of Neuroscience, NIDDK and Lab. of Clinical Studies, NIAAA, National Institutes of Health, Bethesda, MD 20892.

A greater resistance to stress and depression has been associated with increased immunocompetence. Evidence indicates that daily handling of neonatal rats results in an increased ability to cope with stress and in delayed development of both ulcers and tumor growth. In the present study, male and female Wistar rats were handled or left undisturbed during postnatal days 5-20. At 55 days, the time spent immobile in Porsolt's swim test (a putative model of depression) was measured. At 6 months of age, several immune parameters were assessed including natural killer (NK) cell activity and the numbers of splenic B, T, helper/inducer T (CD4+), and suppressor/cytotoxic T (CD8+) cells. The latter measures were assessed with fluorescent monoclonal antibodies against cell surface markers for B (αIgG), T (αThy1.2), CD4+ (αL3T4) and CD8+ (αLyt2) cells.

The duration of immobility in the water was significantly shorter in handled than in control rats ($F(1,36) = 33.5$, $p < 0.0001$). NK cell activity was 40% higher in handled rats ($F(1,26) = 12.7$, $p < 0.02$). Handled rats had fewer T- ($p < 0.001$) and CD8+ ($p < 0.02$) cells than control rats, resulting in a higher CD4/CD8 ratio ($p < 0.05$). The number of B- and CD4+ cells did not differ between treatment groups. These results indicate that early postnatal handling increased both performance in an animal model of depression and certain aspects of immunocompetence.

494.14

I. COMPARTMENT SPECIFIC CELL TRAFFICKING: POTENTIAL MECHANISM FOR STRESS-INDUCED IMMUNOMODULATION. M. Fleshner, L. R. Watkins, L. L. Lockwood, D. Bellgrau*, M. L. Laudenslager & S. F. Maier. DEPT. Psych., U. CO., Boulder, CO 80303

Stressors can alter immune function by many mechanisms. One hypothesis for stress-induced immunomodulation [SII] is changes in cell populations via lymphocyte trafficking. Since immune responses require cooperation of different cell types, stress-induced shifts in cell populations may affect an organism's ability to mount an immune response. We sought to determine if stress could alter lymphocyte trafficking & if so, whether this could be a mechanism for SII. To test for stress-induced lymphocyte trafficking, male rats either remained undisturbed in their home cages or were exposed to 100 1.6 mA inescapable tail shocks. Lymphocytes from peripheral blood, spleen, cervical lymph nodes and superior mesenteric lymph nodes (MLN) were collected and identified as positive for the cell surface markers: CD5, CD8, Ig, & CD4. Flow cytometry revealed a significantly increased $CD4^+/CD8^+$ ratio in MLN of shocked vs. control rats ($p<.001$). In contrast, peripheral blood, spleen, & cervical lymph node cell populations were not measurably altered. If this change in cell populations underlies SII, then immunomodulation should occur only if antigen is processed in the altered MLN. We thus compared the effect of shock on *in vivo* anti-KLH Ig levels after MLN exposure to KLH (by intraperitoneal injection) or spleen (by intravenous injection). Shock induced reductions in serum IgM & IgG levels (ELISA) were indeed observed only following IP KLH. Additional experimentation revealed that: 1) an intact spleen is necessary for stress-induced cell trafficking and Ig reduction, 2) the $CD4^+/CD8^+$ increase is seen in MLN 1 hr. but not 24 hrs. post stress, and 3) SII is not found when IP KLH is given by 48 hrs. post stress. In sum, these data suggest that SII of *in vivo* antibody production occurs due to cell trafficking. Shock seems to selectively mobilize splenic $CD4^+$ cells to move into MLN and $CD8^+$ lymphocytes to move out of MLN, thus altering the immune response to antigen processed at this site. ONR00014-85K0211, BNS-8808840 & MH37373.

494.15

II. COMPARTMENT SPECIFIC CELL TRAFFICKING: NEUROENDOCRINE BASES. L. R. Watkins, M. Fleshner L. L. Lockwood, D. Bellgrau*, M. L. Laudenslager & S. F. Maier. D. Psych., U.CO. Boulder, 80303

Stress can induce changes in immune function. The mechanism(s) for these changes remain unclear. One hypothesis for stress-induced immunomodulation [SII] for which we have evidence (Fleshner, et al., this vol) is changes in lymphocyte populations via cell trafficking. Since immune responses require cooperation of different cell types, stress-induced shifts in cell populations render an organism less able to mount an immune response. We next sought to investigate possible mediators of SI cell trafficking. We focused on 2 systems, the hypothalamic-pituitary-adrenal axis (HPA) and sympathetic outflow. These systems are activated during stress and can influence lymphocytes and lymphoid compartments. Specifically, we sought to determine if adrenal medullary hormones, corticosterone or splenic sympathetic innervation were necessary for stress-induced changes in cell trafficking. Rats were either adrenal demedullated (ADM), treated with intracerbroventricular (ICV) dexamethasone (Fleshner, et al. NS abst, 1989), or sympathectomized. Animals either remained undisturbed in their home cages or were exposed to inescapable tailshock. Blood samples were collected before (BL) and immediately after (100 sk) stress. ACTH and corticosterone levels were determined (RIA) to verify that 1) the adrenal cortex was still functioning in the ADM rats and 2) ICV Dex blocked HPA activation. Lymphocytes from superior mesenteric lymph nodes (MLN) and cervical lymph nodes were collected and identified as positive for the cell surface markers, CD4 & CD8. BL and 100 sk steroid and ACTH levels in ADM shocked rats did not differ from sham operated shocked rats. Shocked rats treated with ICV Dex had lower 100 sk steroid (not different from BL) and ACTH levels than vehicle treated shocked rats. Flow cytometry revealed a significant increase in $CD4^+/CD8^+$ ratio in MLN in ADM but not in ICV Dex treated rats. Thus, HPA activation but not medullary hormones seems necessary for SI changes in cell trafficking. The role of sympathetic outflow is currently being investigated. ONR00014-85K0211, BNS-8808840 & MH37373.

494.16

III. STRESS-INDUCED IMMUNOMODULATION: A STRAIN COMPARISON. L. L. Lockwood, M. Fleshner, L. R. Watkins, M. L. Laudenslager & S. F. Maier. Dept. of Psych., U. CO., CO Boulder, 80303

Stress can induce changes immune function. Since rat strains are known to differ with respect to their responsiveness to stressors, it is possible that stress may have varying immunologic impact on different rat strains. Exposure to inescapable tail shocks produces decreased KLH specific Ig levels and increased $CD4^+/CD8^+$ ratios in Sprague Dawley Holtzman rats (Fleshner et al., this vol). This reduction in Ig levels was seen only when KLH was given immediately prior to shock and not when KLH was given 48, 72, or 96 hrs prior to shock. We thus sought to determine if these changes could also be seen in Fischer 344 rats. Specifically, we investigated if in Fischer 344 rats 1) shock immediately post immunization reduces KLH specific Igs 2) shock produces a compartment specific increase in $CD4^+/CD8^+$ ratio and 3) shock has no effect on anti-KLH Igs when KLH is given 48, 72, or 96 hrs prior to stress. Fischer 344 strain male rats were immunized intraperitoneally with KLH and exposed immediately to 100 1.6 mA tail shocks. Blood samples were collected 3, 5, 7, 9, 14 & 21 days post immunization. Serum levels of KLH specific IgM and IgG were determined using ELISA. As seen in Sprague Dawley Holtzman rats, Fischer 344 strain rats show a profound decrease in both IgM and IgG anti-KLH Igs. To determine if shock induces changes in CD4 and CD8, Fischer 344 rats were sacrificed immediately following shock. Superior mesenteric and cervical lymph nodes were collected and lymphocytes were identified as positive for the cell surface markers CD4 and CD8. Flow cytometry revealed a significant increase in $CD4^+/CD8^+$ ratio specific to mesenteric lymph nodes. Thus, the stress-induced changes reported here seem to be consistant across strains. We are currently investigating whether shock has any effect on Fischer 344 antibody levels when KLH is given prior to shock. ONR00014-85K0211, BNS-8808840 & MH37373.

494.17

SOMNOGENIC IMPACT OF TRYPANOSOMIASIS IN RABBITS. L.A. Toth and J.M. Krueger. University of Tennessee, Memphis, TN 38163.

Trypanosomes cause the disease known as sleeping sickness in humans and induce a chronic wasting disease in rabbits. We examined the somnogenic impact of subcutaneous inoculation of rabbits with trypanosomes. Organisms were first detected in blood 7 days after challenge. Parasitemia correlated to the onset of fever, decreased food intake and elevated fibrinogen levels. The amount of slow wave sleep (SWS) was not markedly altered at this time, but rapid eye movement sleep (REMS) was reduced.

On days 7-17 after inoculation, fluctuations in blood parasite numbers and other physiologic parameters were observed. Daily SWS time gradually decreased below control values, as did delta wave amplitude (DWA) during SWS. The reduction in SWS first developed during the dark period, but eventually occurred throughout the day. However, a circadian pattern of sleep persisted, as did REMS suppression. These reductions in SWS, DWA and REMS reflect major disruptions in sleep architecture that are likely to be related to reduced quality of sleep.

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494.18

THE SUPPRESSANT EFFECTS OF INTERFERON ON ACTIVITY AND FOOD INTAKE ARE NOT PROSTAGLANDIN MEDIATED M.A. Segall and L.S. Crnic, Univ Colorado School of Medicine, Denver, CO 80262

When used to treat cancer, interferon-α (IFN-α) produces fatigue, anorexia, and impaired cognition. A 1600 U/gm, i.p. injection of mouse IFN-α caused a decrease in locomotion and food pellets delivered that lasted 23 hrs after injection (Beh. Neuroscience, in Press). One possible mechanism for the decreased activity and consumption is the release of prostaglandins induced by IFN-α. This was tested by blocking prostaglandin synthesis with indomethacin. DBA/2J male mice (n=32) were placed for 2 days in a chamber with a photobeam activity monitor and automated food pellet delivery. Locomotion and number of food pellets delivered were recorded 24 hr/day. On the 2nd day the mice were given one of four i.p. injections: buffer, indomethacin (5 mg/kg), mouse IFN-α (1600 U/gm), or indomethacin (5 mg/kg) + mouse IFN-α (1600 U/gm). Ifn-α significantly suppressed locomotion and food pellets obtained in the 6 hour period after injection, as previously described, however only the effect on food pellet delivery was significant over the 23 hour period. There was no significant indomethacin main effect on any variable, nor IFN by indomethacin interaction. Indomethacin did not reverse the effects of IFN. The lack of effect of indomethacin suggests that prostaglandins do not mediate the suppressant effects of IFN-α on activity and food intake. Supported by MH09718 & MH00621.

494.19

IMMUNODEFICIENCY AND ACTIVITY-BASED ANOREXIA. P.F. Aravich, L.E. Doerries*, E. Farrar*, S. Downing*, A.H. Elhady*, A. Metcalf* & A.M. Johnson*. Dept. Anat./Neurobiol., Eastern Virginia Med. Sch., Norfolk, VA 23510; Vet. Affairs Med. Ctr., Hampton, VA 23667; Dept. Psych., Christopher Newport Coll., Newport News, VA 23606.

Anorexia nervosa is correlated with various abnormalities including excessive exercise and immunodeficiency. We have been exploring the phenomenon of activity-based anorexia (ABA) in the rat, produced by superimposing voluntary wheel-running exercise on a restricted-feeding schedule, and have found specific opioid abnormalities that are not a secondary consequence of weight loss. These abnormalities could potentially affect immune function, which is reduced in ABA. This experiment determined if ABA immunodeficiency can be distinguished from weight-loss immunodeficiency. Adolescent male rats were subjected to a delayed-type hypersensitivity skin test and sacrificed following a 26% weight loss. Control groups consisted of a weight-loss matched group and an ad lib fed group. Compared to normal rats, ABA and weight-loss matched rats had comparable reductions in induration size, leukocyte number and relative thymus and spleen weights, and comparable increases in relative adrenal weight. We conclude that, on these measures, ABA immunodeficiency is a secondary consequence of weight loss and that ABA-specific opioid abnormalities do not account for the deficits.

494.20

MURAMYL DIPEPTIDE (MDP)-INDUCED PRODUCTION OF TUMOR NECROSIS FACTOR (TNF) BY HUMAN GLIOBLASTOMA CELLS EXPRESSING HLA$_{DR}$. L. Hong*, A.E. Postlethwaite*, L. Johannsen and J.M. Krueger. Univ. of TN, Memphis, TN 38163

In humans, the major histocompatibility complex (MHC) class II molecule HLA$_{DR2}$ is associated with the narcolepsy (1). Astrocytes express HLA$_{DR}$ in response to IFN gamma. These cells also bind MDP and produce TNF; both substances are somnogenic. We investigated if prior treatment with IFN gamma alters the ability of a human glioblastoma cell line, HTB16, to produce TNF in response to MDP stimulation. HTB16 cells were grown with or without IFN gamma. On the 5th day, IFN gamma was removed and the cells were treated with MDP (0.01 - 1 μg/ml) for 2 d. Expression of HLA$_{DR}$ was quantified by ELISA. TNF in culture supernatants from cells was measured using an L cell cytotoxicity assay. IFN gamma-induced expression of HLA$_{DR}$ in these cells was dose- and time-dependent. MDP (0.01, 0.1 ug/ml) enhanced production of TNF in HLA$_{DR}^+$ cells, but not in HLA$_{DR}^-$ cells. The results suggest that the responsiveness of glial cells to MDP depends on prior exposure to IFN gamma. We are now exploring the possibility that enhanced TNF production in HTB16 cells is HLA$_{DR}$ dependent; the TNF gene is located within the region of the MHC suggesting that it is involved in the pathology of HLA-associated diseases.

(1) Juli, T et al. Tissue Antigens. 24:316, 1984

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495.1

FAILURE OF ESTRADIOL (E2) TO INDUCE AN LH SURGE IN OVARIECTOMIZED (OVX) RATS EXPOSED TO CONSTANT LIGHT (LL): EFFECTS OF NALOXONE (NX) TREATMENT. I. R. Cohen and M. R. Sterner, Lilly Research Laboratories, Eli Lilly & Co., Greenfield, IN 46140.

Cyclic release of LH is dependent on the presence of an estrogen stimulus and normal circadian rhythms. While estrous cyclicity is maintained in intact rats following short term exposure to LL, long term exposure leads to a loss of spontaneous LH surges. In addition, the opiate system appears to be involved in modulating LH secretion via effects on neurotransmitter activity. Thus, the effects of short term exposure to LL on the E2-induced LH surge in OVX rats and the role of the opiate system in mediating these effects were examined.

Fischer 344 rats (12 L: 12D photoperiod) were OVX and half were placed into LL (Day 0). Rats received a Silastic implant of E2 on Day 7 and the right atrium was cannulated on Day 8. On Day 9 rats were bled hourly (1200-2000h: 0.5ml). A second group was treated as described but on Day 9 one third of the rats in LL and LD received NX (10 mg/kg BW) at 0915h, NX at 1215h or saline at 0915h and were bled hourly (0900-1700h). LH was determined by RIA.

E2 failed to induce an LH surge in OVX-LL rats. An LH surge was evident in LD E2-treated rats by 1500h and peaked between 1700-1800h. NX at 0915 or 1215h enhanced and advanced the LH surge in LD rats. Also NX at 0915 elevated basal levels of LH at 1000 and 1100h. NX treatment in LL-E2 rats failed to elicit an LH surge. LL rats responded to NX treatment with only a brief elevation in basal LH levels.

These data indicate that: (1) the inhibitory effect of LL on the LH surge is enhanced following OVX and (2) the opiate system does not appear involved in mediating the inhibitory effect of LL on the LH surge mechanism.

495.2

FAILURE OF ESTRADIOL (E2) TO INDUCE AN LH SURGE IN OVARIECTOMIZED (OVX) CONSTANT LIGHT (LL) RATS: EFFECTS OF PROGESTERONE TREATMENT. M.R. Sterner and I.R. Cohen. Lilly Research Laboratories, Eli Lilly & Co., Greenfield, IN 46140.

It has recently been demonstrated that E2 treatment fails to elicit an LH surge in OVX rats housed short-term in LL. Since progesterone (P) exposure has been shown to enhance the magnitude of the E2-induced LH surge in OVX rats, the ability of combined E2+P treatment to restore the LH surge in OVX rats housed in LL was examined.

Fischer 344 rats were bilaterally OVX and housed in 12L:12D (LD) or LL (Day 0). On Day 7 rats received a Silastic capsule (s.c.) containing E2, followed 24h later (Day 8) by right atrial cannulation. On Day 9 blood samples were taken hourly (0.5ml;1200-2000h). Rats in the LL groups received an injection of oil or P (5mg P/0.25ml oil) at 1220h. Serum was assayed for LH and PRL levels by RIA.

E2 treatment alone failed to induce an LH surge in the LL rats. Moreover, combined E2-P treatment also failed to elicit an LH surge in the LL rats. E2 exposure of control LD rats resulted in an LH surge. PRL levels were unaltered in E2-treated LL rats. In contrast to the LH results, E2+P treatment did affect PRL release in LL rats. Combined E2+P treatment of LL rats resulted in a significant increase in PRL from 1600-2000h. E2-treated control LD rats exhibited a PRL surge which peaked at 1500h.

This study demonstrates that : (1) E2 treatment alone is inadequate to alter basal levels of LH or PRL in OVX-LL rats and (2) E2+P is insufficient to induce an LH surge in OVX-LL rats but does result in an increase in afternoon PRL levels. The neurochemical mechanisms mediating these photoperiod effects are currently under investigation.

495.3

COLOCALIZATION OF ESTROGEN RECEPTOR AND GALANIN IMMUNOREACTIVITY IN THE RAT PREOPTIC AREA. K.L. Cashon and R.E. Watson, Jr. Dept. Anatomy and Neurobiology, Univ. of Kentucky Medical Center, Lexington, KY 40536-0084.

Galanin (GAL) immunoreactive (ir) neurons are abundant in the preoptic area and hypothalamus. Bloch, et al. (Soc. Neurosci. Abstr. 15: 577, 1989) have shown that GAL-ir neurons are especially plentiful in important sexually differentiated components of the preoptic area and that many of these neurons concentrate radiolabeled estrogen. This study was conducted to determine if GAL-ir neurons in these sexually differentiated regions express estrogen receptor (ER) immunoreactivity. Intact and gonadectomized female Sprague-Dawley rats were treated with colchicine intracerebroventricularly and perfused with Zamboni's fixative. Frozen sections (25 µm) were incubated in monoclonal antibody H222 (Abbott Laboratories; 10 µg/ml) and reacted using nickel ammonium sulfate-intensified DAB which yielded a blue-black reaction product that was largely restricted to nuclei of immunopositive cells. Sections were then incubated in rabbit primary antiserum to GAL (Peninsula Labs) and reacted with DAB, producing the typical reddish-brown reaction product in the cytoplasm of immunopositive cells. Double-labeled cells, characterized by a blue-black nucleus (ER-ir) surrounded by reddish-brown cytoplasm (GAL-ir) were present in the anterior medial preoptic nucleus (Paxinos and Watson atlas, 1986; corresponding to the anteroventral periventricular nucleus (Simerly and Swanson, Brain Res. 400:11, 1987)), periventricular preoptic area, and the medial preoptic nucleus, especially its central part. Numerous double labeled cells were present in both intact and gonadectomized females. Thus, these results lend further support to the notion that GAL-ir neurons in sexually differentiated components of the preoptic area may serve as targets for estrogen action and contribute to expression of hormonally mediated function. {Supported by the University of Kentucky Medical Center Research Fund.}

495.4

FURTHER EVIDENCE THAT LHRH NEURONS ARE NOT DIRECTLY ESTROGEN RESPONSIVE: LHRH AND ESTROGEN RECEPTOR IMMUNOREACTIVITY IN THE GUINEA PIG BRAIN. R. E. Watson, Jr., M.C. Langub, Jr., and J. W. Landis*. Dept. Anatomy and Neurobiology, Univ. of Kentucky Medical Center, Lexington, KY 40536-0084.

Evidence obtained from steroid autoradiography (Shivers, et al., 1983; in rat) and estrogen receptor (ER) immunocytochemistry (Karsch and Lehman, Soc. Neurosci. Abstr. 14: 1069, 1988; in sheep), has indicated that the LHRH system is not directly estrogen responsive. The present study was conducted in the guinea pig brain to examine the relationship of LHRH- and ER-immunoreactive (ir) cells using a double label immunoperoxidase method. Intact male and female guinea pigs (Hartley strain, young adult, 4 of each sex) were perfused with Zamboni's fixative and 25 µm frozen sections were processed for the sequential localization of ER-ir (H222, Abbott Laboratories) and LHRH-ir (LR-1, Dr. R. Benoit). ER-ir was demonstrated with nickel ammonium sulfate-enhanced DAB as the chromogen which produced a bluish-black reaction product that was largely confined to cell nuclei. LHRH-ir was demonstrated with DAB which produced the typical reddish-brown reaction product in neuronal cytoplasm. The distribution of LHRH- and ER-ir cells overlapped extensively; abundant LHRH-ir neurons and ER-ir cells were located in the medial preoptic area, anterior hypothalamus, area of the tuber cinereum, infundibular nucl., and ventrolateral nucl. (nomenclature from the atlas of Bleier, 1983). However, in the 8 analyzed brains, only a few LHRH cells have been found to also be immunopositive to ER. Frequently, LHRH-ir neurons were located in immediate proximity to many ER-ir cells, which often were arranged in clumps or aggregates. Interestingly, at the most anterior aspect of the preoptic area, abundant varicose LHRH-ir fibers streamed directly past many ER-ir cells, suggesting the possibility of synaptic interactions between them (see Langub, et al., this volume). These results indicate that in the guinea pig the primary estrogenic control of the LHRH system is imparted by estrogen receptor-positive interneurons. {Supported by the University of Kentucky Medical Center Research Fund.}

495.5

SYNAPTIC INTERACTIONS OF LHRH-IMMUNOREACTIVE TERMINALS WITH ESTROGEN RECEPTOR-IMMUNOREACTIVE NEURONS IN THE GUINEA PIG PREOPTIC AREA. M.C. Langub, Jr., B.E. Maley, and R. E. Watson, Jr. Dept. Anatomy and Neurobiology, Univ. of Kentucky Medical Center, Lexington, KY 40536-0084.

During an examination of the relationship of LHRH and estrogen receptor (ER) immunoreactivity (ir) in the guinea pig brain (Watson, et al., this volume), it was noted that LHRH-ir varicosities were frequently in close apposition to ER-ir cells. This study was conducted to examine ultrastructurally the relationship of LHRH-ir terminals and ER-ir cells. Female guinea pigs (Hartley strain) were perfused with buffered 4% paraformaldehyde and 0.1% glutaraldehyde and 50 µm brain sections were processed for the sequential localization of ER- and LHRH-ir. ER-ir was demonstrated with monoclonal antibody H222 (Abbott Labs) using TMB as the chromogen (Norgren and Lehman, 1989). Sections were then incubated in anti-LHRH (LR-1; Dr. R. Benoit) and reacted using DAB as the chromogen. These two chromogens produce reaction products that are readily distinguishable at the ultrastructural level. Thin sections at the rostral pole of the preoptic area at the medial preoptic nucleus (MP; nomenclature from the atlas of Bleier, 1983) and immediately adjacent to it, revealed abundant ER-ir cells that were identified by the presence of crystalline TMB product within nuclei. DAB labeled LHRH-ir processes and terminals were frequently seen in apposition to ER-ir cells. Less frequently, LHRH-ir synaptic terminals contacted ER-ir perikarya. A large number of chemically-uncharacterized synapses were also present on ER-ir perikarya. These results indicate that LHRH neurons can provide input to ER-ir neurons. In light of the fact that LHRH neurons themselves do not express ERs (Watson, et al., this volume, and others) it is likely that ER-ir systems in the preoptic area and hypothalamus regulate the activity of LHRH neurons. The present finding of LHRH synaptic input to ER-ir neurons suggests the existence of a feedback loop in which the activity of ER-positive neurons can in turn be regulated by LHRH - ir neurons. {Supported by the University of Kentucky Medical Center Research Fund.}

495.6

EFFECT OF GONADECTOMY ON GNRH CELLS IN THE NERVUS TERMINALIS AND PREOPTIC AREA IN MALE AFRICAN CICHLIDS, HAPLOCHROMIS BURTONI. **R.C. Francis, R.D. Fernald* and B. Jacobson. Institute of Neuroscience, University of Oregon, Eugene, OR 94703.**

Magnocellular neurons in the preoptic area (POA) that are immunoreactive to GnRH (irGnRH) control the rate of maturation in males of Haplochromis burtoni. The size of these neurons is influenced by the social environment, such that dominant individuals exhibit large soma sizes and subordinate individuals relatively small soma sizes. Here I sought to determine whether androgen feedback is required to maintain the large cells in dominant fish. Subjects were either gonadectomized or sham-operated. After four weeks all fish were sacrificed. Gonadectomized fish were examined for residual testes. Their brains were then removed, sectioned on a cryostat and stained with an antibody to salmon GnRH. Gonadectomized fish showed no reduction in soma size relative to controls, nor any difference in the intensity of the antibody stain. In addition, there was no reduction in aggression in gonadectomized males compared to sham-operated fish. These results suggest that circulating androgens do not modulate soma size in GnRH-secreting neurons of adult males, and that aggressiveness too, is relatively independent of androgen levels.

495.7

SIMULTANEOUS *IN VIVO* MEASUREMENT OF LH AND LHRH FROM THE SAME ANTERIOR PITUITARY PUSH-PULL PERFUSION SAMPLE IN INTACT AND CASTRATED MALE RATS. R.L. Pickle and V.D. Ramirez. Dept. of Physiology and Biophysics, University of Illinois, Urbana, Illinois 61801.

The utility of push-pull perfusion (PPP) for neuroendocrine investigation in conscious animals has recently been expanded in our laboratory with the ability to contemporaneously measure LHRH and LH from the same anterior pituitary perfusion sample. Young adult male rats were stereotaxically implanted with a push-pull cannula in the anterior pituitary 14d prior to experimental use. The animals were perfused with a modified KRP containing 0.1 mM bacitracin at a flow rate of 20 µl/min. Perfusions lasted 4-6h and were continuous for the duration of the experiment with fractions being collected on ice at 10 min intervals. Samples were immediately partitioned for separate LH and LHRH radioimmunoassays, snap frozen on dry ice, and stored at -20°C until time of assay.

Intact (n=3) and 14d castrated (n=3) animals exhibited clearly pulsatile release profiles for LHRH as well as for LH. Detailed analysis of the release profiles for both hormones was performed using the PC Pulsar program. As demonstrated previously, mean LHRH release approximately doubled for castrate animals compared to intact animals, with no significant difference observed in pulse frequency or amplitude between intact or castrate animals. Intact animals exhibited a mean pulse frequency of 1 pulse every 32.5 ± 4.5 min with castrated animals exhibiting 1 LHRH pulse every 28.8 ± 1.1 min. However, LH pulse frequencies did differ ($p<0.01$) between these groups, with observed frequencies of 1 pulse every 53.3 ± 9.6 min and 1 pulse every 32.5 ± 0.9 min for intact and castrated animals, respectively. The disparity between LH and LHRH pulse frequencies within each group, reflects the substantially higher number of silent LHRH pulses (those not temporally associated with a LH pulse) shown by intact animals.

These observations are in agreement with a number of previous reports addressing this issue by sampling circulating levels of LH, and further agree with the notion that the pituitary of intact animals is less responsive to hypothalamic LHRH as a result of testosterone's effect on the gonadotrophs.

495.8

DISTRIBUTION OF ESTROGEN RECEPTOR (ER) CONTAINING AND GnRH NEURONS IN THE RHESUS MACAQUE. K.A. Sullivan, A. J. Silverman, J. W. Witkin and M. Ferin, Depts. Anat. & Cell Biol., & OB/GYN, Columbia Univ. N.Y., N.Y. 10032

Gonadal steroids are known to regulate the synthesis and secretion of GnRH. Colocalization studies using autoradiography for 3H estradiol and immunocytochemistry for GnRH (Nature 304:345,'83) identified very few GnRH cells that contained ER. The present study was undertaken to re-examine this issue using a rat monoclonal antibody (H222, Abbott Labs) to ER and a rabbit polyclonal antibody to GnRH (LR1, Benoit) in the ovariectomized rhesus macaque. Animals were perfused with 4% paraformaldehyde, the brain was fixed overnight and 50um sections through the preoptic area (POA), anterior hypothalamus (AHA) and medial basal hypothalamus (MBH) cut. ER was visualized with a nickel-DAB and GnRH with FITC. Cells immunoreactive for ER were observed within the medial preoptic area as well as periventricular, ventromedial and infundibular nuclei. ER immunoreactivity was mainly confined to nuclei of cells but was also present in the cytoplasm of a few neurons. GnRH neurons were observed in the preoptic area as well as in hypothalamic nuclei. (JCN, 211:309,'82). Many of these neurons were fusiform in shape and extended long dendrites, while a few were multipolar. The distributions of GnRH neurons and ER cells did not overlap and were rarely found close together. Of the 293 GnRH neurons counted to date, none contained ER. Experiments are currently underway to determine the neurotransmitter phenotype of the ER-containing cells and how they might relate synaptically to GnRH neurons.DK 42323

495.9

GABA RECEPTOR ANTAGONISTS INCREASE LHRH NEURONAL RESPONSIVENESS TO NOREPINEPHRINE (NE). R.Hartman, J. He* and C.A.Barraclough,Dept of Physiol, Sch. Medicine, Univ. of Maryland, Baltimore, MD 21201.

These studies examined whether the limited responsiveness of LHRH neurons to norepinephrine (NE) we observed in previous studies was due to the endogenous secretion of GABA from local hypothalamic interneurons. The icv or medial preoptic area (MPOA) infusions of either bicuculline (BIC; GABA-A antagonist) or phaclofen (PHAC; GABA-B antagonist) into chloral hydrate anesthetized, ovariectomized, estrogen-treated rats did not affect basal LH levels (95 ± 8.5 ng/ml). When NE was infused icv, it produced a modest rise in plasma LH which peaked within 15 min (240 ± 25 ng/ml). In contrast, if BIC (2 ng/2 µl) or PHAC (20 ng/2 µl) was given icv and NE (45 ug/2 µl) was infused 15 min later, plasma LH was markedly increased to peak levels of 723 ± 98 and 844 ± 126 ng/ml, respectively, within 15 min after icv NE. Similarly, when either BIC (0.5 ng/0.5 µl) or PHAC (10 ng/0.5 µl) was infused into the MPOA and NE was given 15 min later (icv) peak LH levels of 726 ± 105 and 844 ± 126 ng/ml, respectively, were obtained.

Seemingly, LHRH neurons, in their normal resting state (e.g. when plasma LH levels are low), are relatively unresponsive to NE and this may be due to the endogenous secretion of GABA from local inhibitory interneurons located within the MPOA. NIH Grant HD-02138.

495.10

IN-VITRO RELEASE OF LHRH AND 5-HIAA/5-HT FROM THE MEDIAL-BASAL-PREOPTIC HYPOTHALAMUS (MBH) OF OVARIECTOMIZED (OVX) E_2 TREATED AND PROESTROUS RATS: EFFECT OF GABA STIMULATION. R.C. McRee* and D.C. Meyer, Dept. of Physiology, Eastern Va. Med. School, Norfolk, VA 23501.

Studies on female rats suggest that 5-HT modulates LH release through LHRH systems while a GABA-A receptor-mediated mechanism can increase LHRH release in male rats. Since GABA is a major neurotransmitter in the CNS the following experiments utilized an in-vitro superfusion system (Krebs-Ringer-Phosphate) to measure the effect of GABA on LHRH and 5-HIAA/5-HT (index of 5-HT activity) release from the MBH of proestrous and OVX E_2 treated rats. MBH tissue was removed at 1000 Hours (0500 - 1900 H. light), and 500 µl. fractions were collected (50 µl/min.) and assayed for 5-HT and 5-HIAA by HPLC-EC and LHRH by RIA. The release of LHRH was significantly decreased ($P<.05$) following GABA superfusion (1×10^{-4} M) in OVX E_2 treated rats, however GABA had no significant effect in proestrous rats. GABA had no significant effect on the 5-HIAA/5-HT ratio in the OVX E_2 treated rat but significantly decreased the 5-HIAA/5-HT ratio on proestrus ($P<.05$). Collectively these data suggest that both 5-HT and GABA may play a role in LHRH release and that GABA's actions are dependent on the level of E_2 and may not utilize 5-HT systems.

495.11

LUTEINIZING HORMONE (LH) PULSE FREQUENCY, BUT NOT AMPLITUDE IS REDUCED FOLLOWING INHIBITION OF DOPAMINE-β-HYDROXYLASE (DBH). S.J. Legan*, J.H. Urban, S.S. Mehta*, L.A. Conaghan*, J.E. Levine. Dept. Neurobiol & Physiol, Northwestern Univ., Evanston, IL 60208; *Dept. Physiol & Biophysics, Univ. of Kentucky, Lexington, KY 40536.

We recently demonstrated that α_1-adrenergic receptor blockade reduces frequency but not amplitude of pulsatile LH secretion. This study examined the effects of reduced catecholaminergic (CA) neurotransmission on LH pulse frequency and amplitude. 6d castrate or 14d ovariectomized rats were bled through atrial catheters at 5min intervals for 2h. Rats received an i.v. injection of the DBH inhibitor FLA-63 (25mg/kg) or vehicle (50% propylene glycol). Sampling was resumed 2-4h following injections. Treatment with FLA-63 decreased LH pulse frequency in male (3.6±0.1 to 0 pulses/h,n=3) and female rats (3.3±0.5 to 0.8±0.3 pulses/h,n=6; $p<.02$). Mean pulse amplitude in the females was unchanged (2.46±0.21 ng/ml versus 2.60±0.47 ng/ml). Pulse parameters were unaffected by vehicle treatment. These data demonstrate that suppression of noradrenergic/adrenergic transmission reduces the activity of the LHRH pulse generator without affecting LH pulse amplitude. Our observations suggest that CA facilitation of LH secretion is exerted on the pulse generating mechanism only and not on other cellular processes modifying LHRH secretion. Moreover, the FLA-63-treated rat provides a pharmacological model in which pulse generator activity is selectively suppressed for a prolonged period, thereby allowing for detailed analyses of pulse generator regulatory mechanisms. (NIH HD20677, HD21921, HD00879, JEL; HDo7304, SJL).

495.12

OVARIAN HORMONES INCREASE DOPAMINE RECEPTOR CONCENTRATION IN THE HYPOTHALAMUS AND STRIATUM OF THE RAT. J. F. Rodriguez-Sierra and L. C. Murrin. Departments of Anatomy and Pharmacology, University of Nebraska Medical Center, Omaha, NE 68198

Prepuberal rats (25-days of age) were treated with either estradiol benzoate (EB, 10 µg, sc) or sesame oil vehicle at 1200h. Forty four hours later some of the animals were treated with progesterone (PROG, 1 mg, sc) or with sesame oil vehicle. Animals were sacrificed either at 900h or at 1600h at 27 days of age. The brains were removed and 1 mm thick sections were made using the Jacobowitz brain blocker. The striatum and the arcuate-median eminence regions were microdissected and frozen in dry ice. We pooled three animals for each point in the assay. Tissue was weighed and processed for binding to D1 and D2 receptors as described previously. We used as ligands ^{3}H-SCH-23390 and ^{3}H-spiperone (+ 50 nM ketanserin) for the D1 and D2 receptors, respectively. We found no significant differences between male and female hypothalami in the morning or afternoon at 27 days of age. Administration of EB alone did not alter the concentration of D1 receptors in either sex, but animals administered progesterone after EB showed a significant increase of D1 receptor binding in both male and female rats. The striatal D1 receptors showed a decrease in binding during the afternoon in the female rats. The males did not show this effect in D1 striatal receptors. There was no effect of EB treatment on either sex in the D1 striatal receptor, but the animals administered PROG after EB showed a dramatic increase in binding. We found a significant increase of binding to the D2 receptor in the afternoon for the female, but not the male, rat. The increase in D2 receptor binding in the hypothalamus was augmented by EB treatment and amplified further by PROG administration in the female rat. The male rat did not show the effect of EB, but PROG had a dramatic effect in increasing D2 binding to the D2 receptors in the hypothalamus. The D2 receptor binding in the striatum did not show the afternoon increase in the female rats, but did show a significant increase in binding to the administration of EB and of EB and PROG. The male rats showed an increase of striatal D2 binding after EB and PROG. Our results suggest that ovarian hormones act not only in the tuberoinfundibular hypothalamic area, but also in the striatum to modulate dopamine neurotransmission. We believe that the modulation of the tuberoinfundibular receptors by the ovarian steroids is related to the effects of dopamine on prolactin. The striking effect of progesterone in the female rat striatum might be related to the sterotypic behavior displayed by the female in estrus: such as the head tremor (sometimes called ear-wiggling) and the hopping behavior exhibited when the female is pursued by the male. (This work was supported by grants from the NIH HD-13219 to J.F. R.-S. and NS-23975 to L.C. M.)

495.13

HYPOTHALAMIC POMC mRNA LEVELS DURING RECOVERY FROM CHRONIC ESTRADIOL: DOPAMINERGIC MECHANISM? D.D. Rasmussen, M. Jakubowski, D.L. Allen, J.L. Roberts. Dept Repro Med, UCSD, La Jolla, CA 92093, and Fishberg Res Ctr for Neurobiol, Mt Sinai Sch Med, New York, NY 10029.

Dopamine (DA) appears to regulate hypothalamic POMC-containing neurons. To investigate this regulation we used a model in which rat mediobasohypothalamus (MBH) dopaminergic, but apparently not noradrenergic, activity has been demonstrated to be suppressed by chronic high estradiol (E_2) and then rebound in the weeks following removal of the E_2, surpassing pre-suppression levels. Under at least some steroidal conditions MBH dopaminergic activity is higher in the morning than afternoon, so we also addressed possible diurnal modulation. Rats were ovariectomized (OVX), OVX with 3 d low (18 pg/ml) E_2 , OVX with 20 d high (99 pg/ml) E_2, or OVX with 20 d high E_2 followed by removal of the E_2 for 10 or 20 d. 6 rats/trtmnt were sacrificed at 0900 h and 1500 h and hypothalamic *cytoplasmic* and *nuclear* POMC mRNA were quantified in solution hybridization/-nuclease protection assays. 0900 h levels of *cytoplasmic* mRNA were suppressed 21% (relative to OVX) by chronic high E_2 trtmnt but rebounded 52% by 10 d post-E_2 to a level greater than OVX. 1500 h levels were suppressed 31% by chronic E_2 and then gradually increased 68% to a level greater than OVX by 20 d post-E_2. 0900 h *nuclear* mRNA levels were also suppressed 22% by chronic E_2 and then rebounded 33% 10 d post-E_2, whereas 1500 h levels were increased only after 20 d. At 10 d post-E_2 both *cytoplasmic* and *nuclear* POMC mRNA levels were greater at 0900 h than at 1500 h. The currently demonstrated suppression and subsequent rebound and "overshoot" in POMC mRNA content in response to administration and removal of chronic high E_2 parallels the changes previously demonstrated in MBH dopaminergic activity. We are further investigating this correlation between MBH dopaminergic and POMC/endorphinergic activity by evaluating changes in tyrosine hydroxylase mRNA content in these same samples.

495.14

ESTRADIOL MODULATES THE ACTIVITY AND QUANTITY OF TYROSINE HYDROXYLASE IN THE MEDIAN EMINENCE. C. Pasqualini*, B. Guibert*, N. Faucon-Biguet *, B. Kerdelhué°° and V. Leviel*. L.P.N.- °N.B.C.M. CNRS, 91198 Gif-sur-Yvette, °°Lab. Neurobiol. Reprod. CNRS-INRA 78350 Jouy-en-Josas (France).

The nature and mechanism of estradiol (E2) effects on tuberoinfundibular dopaminergic (TIDA) neurons are still controversial. In the present study, we investigated the effects of an acute E2 treatment in parallel on the activity of tyrosine hydroxylase (TH) in the median eminence (ME) and on serum prolactin (PRL) release. Twelve-days ovariectomized rats were injected with 17βE2 (25µg s.c.) at 11.00h and sacrified hourly from 12.00 to 19.00h. TH activity was quantified by measuring the amount of exogenous tyrosine converted to L-DOPA in vitro by ME homogenates and serum PRL by RIA. A bimodal response to treatment was observed : an immediate decrease in TH activity occurred -preceeding and accompanying a rise in serum PRL- followed by an increase beyond control levels, one hour after the maximal release of PRL. This secondary increase in TH activity was no longer observed when rats were previously treated with a specific rat PRL antiserum, suggesting it was not due to E2 per se , but rather mediated by the E2-induced PRL elevation. To pinpoint the process underlying the E2-induced decrease in TH activity, we also evaluated the kinetic parameters of TH in the ME as well as its quantity (by "Western blot" analysis). The decrease in TH activity after E2 treatment nicely paralleled an immediate decrease in the affinity of TH for its cofactor (6-MPH4), while Vmax was unchanged. A decrease in the amount of TH was also observed but its latency precluded its major involvement in the immediate decline of TH activity. Therefore, when observed independently from those of PRL, E2 effects on TH in TIDA neurons are clearly inhibitory. They consist in a desactivation of the enzyme together with a reduction of its synthesis.

495.15

EVIDENCE FOR INDEPENDENT α_1 ADRENERGIC AND OPIOIDERGIC REGULATION OF PULSATILE LUTEINIZING HORMONE (LH) SECRETION. K. M. Vogelsong and J. E. Levine. Dept of Neurobiology and Physiology, Northwestern University, Evanston, IL 60208

A prevalent hypothesis holds that catecholaminergic (CA) neurons mediate opioidergic inhibition of LHRH release. To test this theory, we analyzed effects of opiate and α_1 adrenergic blockade on pulsatile LH secretion in intact and ovariectomized (OVX) rats. Effects of separate or combined treatments with the opiate receptor antagonist, naloxone (NAL), and the α_1 adrenergic antagonist, prazosin (PRA) were examined to determine (i) which parameters of LH secretion are altered by each drug, and (ii) if α_1 adrenergic blockade eliminates opioid inhibitory tone. Metestrous (MET) or OVX rats with atrial catheters were bled every 5min for 4h. After 1h, rats received PRA (1mg/kg ip) or vehicle, followed by NAL (5mg/kg iv) or vehicle. Frequency, but not amplitude, of LH pulses was suppressed ($p<.05$) by PRA in OVX animals, while the drug was without effect in MET rats. By contrast, NAL increased ($p<.05$) mean LH release in both MET and OVX rats. PRA pretreatment did not block NAL effects in MET rats. In PRA-treated OVX rats, NAL appeared to augment LH secretion in many cases where pulses occurred within 1h following NAL. These data suggest that opioid effects are not mediated exclusively by NA pathways. Instead, it is proposed that opioid inhibitory tone is exerted independently from α_1 adrenergic facilitation of the LHRH pulse generator. (NIH RO1-HD20677, KO4-HD00897, PO1-HD21921).

495.16

SIMULTANEOUS ESTIMATION OF ADRENALINE AND NORADRENALINE TURNOVER IN THE PREOPTIC AREA AND MEDIAN EMINENCE OF PRO-OESTROUS AND DIOESTROUS RATS. J. Opacka-Juffry* and C.W. Coen. Division of Biomedical Sciences, King's College, London, UK.

The present study was designed to assess the simultaneous rates of turnover of noradrenaline and adrenaline in the preoptic region containing the luteinizing hormone releasing hormone (LHRH) cell bodies and in the median eminence, the site of the LHRH terminals. Rats were maintained with lights on from 06.00 to 20.00h. The turnover rates were assessed by catecholamine decline 2 hours after dopamine β-hydroxylase inhibition with 10mg FLA 63/kg given ip at 12.00 or 17.00h on dioestrus and 12.00, 15.00 or 17.00h on pro-oestrus. Samples were micropunched from 300µm cryostat sections which were subsequently fixed, stained and assessed. Although no significant change was found in adrenaline turnover in the preoptic area or median eminence within diestrus, there was a significant increase on pro-oestrus in both sites by the latter sampling point; this time corresponds to that of the LH surge in our colony. Noradrenaline turnover in the same material failed to demonstrate significant changes in the preoptic area within either of the days, but at 12-14.00h on pro-oestrus it was significantly higher than at the same time on dioestrus. While recognizing the limitations of assessing turnover by amine depletion, these results on histologically verified samples suggest that the occurrence of the LH surge is associated with increased adrenaline turnover in the region of the LHRH-containing perikarya and terminals.

495.17

DIFFERENTIAL LH AND FSH RESPONSES TO N-METHYL-D-ASPARTATE IN INTACT AND CASTRATE MALE RATS. F.J. Strobl, U. Luderer*, N.B. Schwartz, and J.E. Levine. Dept. of Neurobiology and Physiology, Northwestern Univ., Evanston, IL 60208.

The excitatory amino acid agonist N-methyl-D-aspartate (NMDA) stimulates LH secretion by evoking an acute increase in LH-releasing hormone (LHRH) secretion. These studies were conducted to determine (i) whether FSH secretion is also stimulated by NMDA treatment, (ii) if any NMDA effects on FSH are mediated by LHRH, and (iii) if LH and FSH responses to NMDA are affected by changes in endocrine status. Castrate (n=10) and sham-castrate (n=11) male rats received 100ug LHRH antagonist or oil vehicle at 2100h and on the next day were bled at 10min intervals through atrial catheters from 0900-1200h. Animals received 5 mg NMDA after 1h and 500ng LHRH after 2h. Plasma LH and FSH were determined by RIA. In oil-treated sham-castrates LH levels were increased by 110% ($p<.01$), while FSH levels were unchanged following administration of NMDA. LH responses to NMDA were attenuated in castrates compared to sham-castrates. FSH levels were also unchanged following NMDA in castrates. LHRH antagonist pretreatment blocked LH responses to NMDA, as well as LH and FSH responses to LHRH. Our data indicate that the amount of LHRH released during the stimulation of an LH pulse is an inadequate stimulus for pulse-like stimulation of FSH secretion. While pulsatile LHRH stimulation may support basal FSH secretion, pulse-like increments in FSH secretion may either be constitutive endocrine events or be driven by a separate FSH-RH. Attenuation of NMDA-induced LH secretion in castrates may reflect a diminishment of the releasable LHRH pool following removal of gonadal negative feedback. Supported by NIH P01-HD21921 and R01-HD20677.

495.18

DIFFERENTIAL GONADOTROPIN RESPONSES TO N-METHYL-D-ASPARTATE (NMDA) IN PROESTROUS (PRO), METESTROUS (MET) AND OVARIECTOMIZED (OVX) RATS. U. Luderer*, F.J. Strobl, J.E. Levine, N.B. Schwartz. Dept of Neurobiology and Physiology, Northwestern Univ, Evanston, IL 60208.

NMDA, an analog of the excitatory amino acid aspartate, elicits luteinizing hormone (LH) and prolactin (PRL) release in rats. These experiments were carried out to i) verify that NMDA-stimulated LH secretion is mediated by LH releasing hormone (LHRH), ii) assess the degree to which NMDA stimulates follicle stimulating hormone (FSH) in a LHRH-dependent manner and iii) analyze the relationship between endocrine status and responsiveness to NMDA. Estrous, diestrous or 8-day OVX rats were fitted with atrial catheters and injected with 100 ug LHRH-Antagonist (Antag) or vehicle at 2100h. Starting at 0900h the next day blood was withdrawn every 10 min for 3h. Each animal received 5 mg NMDA after the first, and 500 ng LHRH after the second, hour. NMDA significantly increased LH in MET and PRO females. Antag reduced the increases. In OVX females LH paradoxically decreased after NMDA. FSH was not affected by NMDA and was slightly suppressed by Antag. PRL increased after NMDA in all groups. LHRH caused surge-like LH and small FSH increases in vehicle groups. These results demonstrate that LHRH mediates the LH response to NMDA in rats; lack of FSH responses to NMDA suggests that physiological increments in endogenous LHRH secretion are insufficient to stimulate pulse-like FSH release. Comparison of NMDA responses also suggests that the releasable pool of LHRH is not increased before the preovulatory LH surge. (Funded by NIH HD07504).

495.19

COMPARISON OF LH AND PROLACTIN RESPONSES TO KAINATE AND NMA IN LACTATING AND CYCLING RATS. R. Abbud* and M.S. Smith. Department of Physiology, University of Pittsburgh School of Medicine, Pittsburgh, PA 15261.

We have reported that in cycling rats, NMA (20-40 mg/kg, iv) stimulates both LH and prolactin (PRL) release (Endocrinology 124: 1905, 1989). In lactating rats, GnRH neurons are refractory to NMA, but NMA inhibits suckling-induced PRL secretion. NMA's effects on PRL, whether stimulation of PRF's or DA predominate, appear to depend on the reproductive state of the animal. Recent autoradiographic studies suggest that kainate receptors may be more abundant than NMDA receptors in the hypothalamus. Thus, we have examined LH and PRL responses to kainate (1.5-2.5 mg/kg iv). Three pulses of kainate were infused 2 hrs apart and serial blood samples were collected at 10 min intervals. Lactating rats suckling 8 pups (day 10 postpartum) were primed for 24 hrs with GnRH pulses (5 ng iv every 50 min) to restore pituitary GnRH receptors before administration of kainate.

In diestrous and estrous rats, kainate induced larger LH responses than did NMA. Surprisingly, PRL secretion did not increase in response to kainate. In the lactating rats, kainate had similar effects as those of NMA, that is, it was unable to overcome the lactation-induced suppresion of LH release, but it did inhibit suckling-induced PRL secretion. By 24 hrs after pup removal, both kainate and NMA induced small increases in LH secretion but only NMA stimulated PRL release.

These data demonstrate that the suckling stimulus induces GnRH neuronal refractoriness to exogenous stimuli such as excitatory amino acids. Also, kainate and NMDA receptors appear to have different distributions on neurons that affect PRL secretion. (NIH grant HD 14643)

REGULATION OF AUTONOMIC FUNCTION: TEMPERATURE REGULATION AND NEURAL-IMMUNE SYSTEM INTERACTIONS

496.1

BROWN ADIPOSE TISSUE RESPONSES OF CONSCIOUS AND ANESTHETIZED RATS FOLLOWING VMH ELECTRICAL OR INTRAVENOUS NOREPINEPHRINE STIMULATION. J.A. Thornhill and I. Halvorson. Dept. of Physiology, Univ. of Saskatchewan, Saskatoon, Sask., Canada S7N 0W0.

Thermoregulatory experiments were conducted in conscious and urethane anesthesized male Sprague-Dawley rats previously acclimated for 3 weeks at 4°C or 21°C to determine if VMH electrical stimulation or intravenous (iv) norepinephrine infusion could activate interscapular brown adipose tissue (BAT) thermogenesis, as indicated by significant increases in IBAT temperature (T_{IBAT}) above core, as measured colonically (T_C). Experiments were conducted twice when the animal was conscious but restrained (trained to Plexiglass holder beforehand) and lastly when anesthetized with urethane (1.5 g/kg ip). T_C, T_{IBAT} and tail (T_t) temperatures and blood pressure (from indwelling femoral arterial catheter) were monitored before and for 20 min after bipolar electrical stimulation (0.5 msec pulses of 100 μA at 50 Hz for 30 sec) of the ventromedial hypothalamic nucleus (VMH) or after intravenous infusion (100 μl/min) of sterile saline and norepinephrine HCl (NE, 20 μg/ml). Conscious or anesthetized rats acclimated to 4°C evoke significant increases in T_{IBAT} above T_C after VMH electrical or iv NE stimulation.

Supported by the Medical Research Council of Canada.

496.2

VENTROMEDIAL HYPOTHALAMIC STIMULATION OF BROWN ADIPOSE TISSUE THERMOGENESIS. L. Kelly* and C. Bielajew, Dept. of Psychology, University of Ottawa, Ottawa, Canada, K1N 6N5.

The ventromedial hypothalamus (VMH) is implicated in the central regulation of brown adipose tissue (BAT) facultative thermogenesis, although inconsistencies exist in the reported observations of effective areas. In this study, VMH stimulation of BAT thermogenesis was mapped with moveable electrodes, lowered in 0.16mm increments. Seven male hooded rats were implanted in the VMH region, with interscapular BAT and core temperatures monitored continuously. Each train of 25 (100μsec) square wave cathodal pulses was delivered at 300μA, 500msec on/500msec off, for a trial duration of 60sec. A mid-VMH descent yielded two active sites, both with a typical BAT temperature change of 0.4-0.6°C, in contrast to the temperature profiles found at the single active site in each of two mid-anterior VMH, and one dorsal medial hypothalmic (DMH) placements. These profiles showed larger temperature changes of 1.1-3.3°C, and slower returns to baseline (70-180min). Non-responsive placements included the anterior and posterior VMH, and two descents immediately lateral to the VMH. A regulatory connection of the VMH to BAT thermogenesis is demonstrated, with the possibility that several different substrates stimulate BAT activity. These could be related to the known differences between cold and dietary signals which both act on BAT through the VMH. Supported by NSERC grant #U0514 to C.B.

496.3

PROSTAGLANDIN-E_2 STIMULATION OF BROWN ADIPOSE TISSUE (BAT) THERMOGENESIS IN SPONTANEOUSLY HYPERTENSIVE (SHR) AND WKY RATS. S.Bhatnagar, M.J.Meaney and S.Amir. Ctr. for Studies in Behav. Neurobiol.,Dept. of Psych., Concordia Univ.,Douglas Hospital Res. Ctr., McGill Univ., Montreal, Canada.

SHRs exhibit diminished ability to maintain body temperature in the cold, but the pathophysiological mechanism responsible for this deficiency is unknown. We studied the possibility that failure of SHRs to defend body temperature in the cold is related to a reduced ability to centrally activate adaptive thermogenesis in BAT. In the present study, injection of PGE_2 (50-200ng) into the POAH had a differential effect on intrascapular BAT temperature (IBATt) of adult, urethane-anesthetized SHRs and normotensive WKYs. The 50ng dose produced an increase of 0.72 ± 0.17°C in IBATt in SHRs compared to a larger increase of 1.65±0.15°C in WKYs ($p<0.01$). 100ng of PGE_2 produced an increase of 1.22±0.26°C in SHRs and an increase of 1.90±0.08°C in WKYs ($p< 0.05$). 200ng of PGE_2 increased IBATt by 1.45±0.23°C and 1.97±0.16°C in SHRs and WKYs, respectivley. It has been shown that injection of PGE_2 into the POAH mimics the effect of cold in stimulating BAT thermogenesis. The finding that SHRs exhibit reduced sensitivity to the central effects of PGE suggests that the diminished ability of these animals to defend body temperature in the cold may be related, at least in part, to a defect in central mechanisms mediating the effect of cold on nonshivering thermogenesis in BAT.

496.4

THE INFLUENCE OF ONE-KIDNEY RENOVASCULAR HYPERTENSION ON THE HYPERTHERMIC RESPONSE TO CENTRAL PGE_1 IN THE RAT. D.M.Fyda, W.L.Veale & Q.J.Pittman, Department of Medical Physiology, University of Calgary, Calgary, Alberta, Canada T2N 4N1.

There is evidence for high circulating levels of arginine vasopressin (AVP) during development of one-kidney/one-clip (1K1C) hypertension. Arginine vasopressin is also known to act centrally as an endogenous antipyretic against a prostaglandin E_1 (PGE_1) fever. As peripheral and central release of AVP has been correlated, this study assessed whether 1K1C hypertension (left nephrectomy followed by the application of a 0.2 mm diameter clip to the right renal artery) influenced the hyperthermia evoked by an intracerebroventricular injection of 150 ng PGE_1 (in 5.0 μl). Remote monitoring of core temperature (Tc) occurred prior to clipping and on days 4, 8 and 12 post-clipping for a 60 min baseline and a 120 min post-PGE_1 injection period. Prior to clipping, PGE_1 evoked a 1.4°C rise in Tc relative to baseline values ($p<.0001$) in all 16 rats. Post-clip, nephrectomized-only controls remained normotensive and continued to show similar rises in Tc following PGE_1. On days 4 and 8 post-clip, the blood pressure of the 1K1C rats steadily increased (as monitored by tail plethysmography) but the PGE_1-evoked rise in Tc was essentially abolished relative to pre-clip levels ($p<.0001$). Blood pressure of the 1K1C rats stabilized on day 12 post-clip at 185 ± 5 mmHg and Tc now rose by 0.84°C ($p<.0001$) following PGE_1. To establish if the suppression of fevers observed during the development of hypertension was due to the central release of AVP, other 1K1C rats received an AVP receptor type 1 antagonist (Manning Compound, 200 pmol in 1 μl bilaterally into the ventral septal area) 15 min prior to PGE_1. Under these conditions the typical hyperthermia was reinstated. These data suggest that during the development of this model of hypertension there may be an increase in the central activity of AVP which contributes to the inability of the animals to obtain a fever. When the hypertension stabilized, the animals were able to obtain a fever, suggesting that central AVP activity had returned towards normal levels. Supported by the MRC (Canada), Heart Foundation (Canada), & AHFMR (Alberta).

496.5

HIGH DENSITY OF PROSTAGLANDIN E_2 BINDING SITES IN THE ANTERIOR WALL OF THE THIRD VENTRICLE (A3V). K. Matsumura[1], Y. Watanabe[*2], H. Onoe[*2], Y. Watanabe[*2], O. Hayaishi[*2]. [1]Dept. of Physiol., Osaka Univ. Med. Sch., Kita-ku, Osaka 530, [2]Dept. of Neuroscience, Osaka Bioscience Institute, Suita, Osaka 565, JPN.

Prostaglandin E_2 (PGE_2) exerts potent hyperthermic action when injected into the preoptic-hypothalmic region (POHA). To determine the exact functional sites of PGE_2, we used in vitro autoradiography of ^{3}H-PGE_2 binding sites in the rat POHA. Frozen sections of rat brain were cut in a cryostat and mounted on glass slides. They were preincubated in Tris-HCL buffer and then incubated with 20 nM ^{3}H-PGE_2 solution for 30 min. Non-specific binding was obtained using consecutive sections by the addition of 100 uM unlabeled PGE_2 to the incubation mixture. They were dried and juxtaposed to ^{3}H-sensitive films. After 4-5 weeks, the films were developed and the optic density was measured. The highest density of ^{3}H-PGE_2 binding was found in the A3V. Within A3V, the density was especially high in regions closest to the third ventricle or surrounding the organum vasculosum laminae terminalis (OVLT) but was relatively low in the OVLT itself and the medial preoptic area/ anterior hypothalmic area. A3V seems to be involved in PGE_2 induced hyperthermia.

496.6

SENSITIVITY OF RAT MEDIAN PREOPTIC NEURONS TO TEMPERATURE, ANGIOTENSIN II AND NOREPINEPHRINE. K.A. Travis and A.K. Johnson. Depts. of Psychology and Pharmacology and the Cardiovascular Center, Univ. of Iowa, Iowa City, IA 52242.

The median preoptic (MnPO) nucleus receives angiotensinergic projections from the subfornical organ, noradrenergic projections from the brainstem and has reciprocal connections with the medial preoptic nucleus of the hypothalamus. The present study sought to determine the specificity of MnPO neuronal responses to angiotensin II (AII) (10^{-4} or 10^{-5}M), norepinephrine (NEpi) (10^{-4} or 10^{-5}M) and changes in temperature (32-42°C).

In-vitro neuronal activity was recorded from MnPO neurons in sagittal tissue slices (350-450 um) of male Sprague-Dawley rats. Single units were evaluated for responses to changes in perfusate temperature and perfusion by AII, NEpi or both AII and NEpi.

Of the neurons tested for sensitivity to changes in temperature (n=40), 75% were thermo-insensitive and 25% were warm sensitive. Of the neurons tested for responses to the drug challenges (n=31), 42% responded to at least one treatment. Most neurons which responded to the drug challenges did so with increased activity (86%). In addition, some neurons showed an additive or synergistic response to AII and NEpi applied together. Drug treatment rarely (1/15) affected the thermosensitivity of the neurons.

496.7

ALTERED BRAIN [^{3}H]IDAZOXAN BINDING IN RABBITS DURING FEVER. L.K. Vaughn. Marquette Univ. Sch. of Dentistry, Milwaukee, WI 53233.

The neuronal mechanisms responsible for fever are incompletely understood and the possible role of changes in the number and/or affinity of brain receptors to thermoregulatory neurotransmitters during fever is unknown. We investigated whether fever is associated with changes in α-2 adrenergic receptors (α-2 AR) since norepinephrine has been implicated as a neurotransmitter involved in fever and α-2 AR are altered by microwave-induced hyperthermia (Gandhi and Ross, Radiat. Res. 109:90,1987). Rabbits were sacrificed 0.5 hr to 4 hr after injection with 0.25 μg/kg Salmonella abortus equi endotoxin (n=5 at each time point) or saline (n=10) and binding of [^{3}H]idazoxan, an α-2 AR antagonist, in tissue homogenates of various brain regions was performed using the method of Reader et al. (Neurochem. Res. 11:9, 1986). Maximal changes in binding occured 2 hr after injection. Further experiments at 2 hr (n=7 saline, n=7 endotoxin) revealed an increase in Bmax in the posterior hypothalamus (PH)(19%) and striatum (STR)(62%). There was also a significant increase in Kd (PH, 25%; STR, 97%). There were no significant changes in the anterior hypothalamus, medulla or cortex. The increased receptor number and decreased receptor affinity might indicate that two populations of α-2 AR are undergoing changes. The observed changes in α-2 AR number and affinity may serve to enhance or modulate regulatory mechanisms involved in fever.

496.8

PERFUSION OF ARGININE VASOPRESSIN WITHIN THE MEDIAL AMYGDALOID NUCLEUS ATTENUATES PROSTAGLANDIN HYPERTHERMIA IN THE URETHANE-ANAESTHETIZED RAT. P. Federico, T.J. Malkinson*, Q.J. Pittman and W.L. Veale. Neuroscience Research Group, The University of Calgary, Alberta, Canada T2N 4N1.

The neuropeptide arginine vasopressin (AVP) has been shown to act within the ventral septal area (VSA) in conscious animals as an endogenous antipyretic. AVP content in amygdalar nerve terminals has recently been found by other investigators to change in febrile or pyrogen tolerant animals, suggesting that AVP may also act in association with fever in this site. Thus, experiments were undertaken to determine whether AVP push-pull perfusion within the medial amygdaloid nucleus (meA) or VSA would attenuate prostaglandin (PGE) hyperthermia in the urethane-anaesthetized rat. The meA was chosen as the novel target for AVP perfusion due to its strong vasopressinergic input as well as its extensive reciprocal connections with brain loci important in fever and antipyresis. Guide cannulae bilaterally directed toward the lateral cerebral ventricles as well as the meA or the VSA were stereotaxically implanted in 66 male Wistar rats (270 -330 g) under urethane anaesthesia (1.5 g/kg). After surgery, the rats were allowed a 30 minute settling period during which colonic temperature, recorded by a thermistor probe, was maintained at a baseline of 37.17 ± 0.05°C. Following this, aCSF or AVP (6.5 μM in aCSF) was bilaterally push-pull perfused within the meA or VSA for 120 minutes prior to an i.c.v. injection of PGE_1 and for an additional 120 minutes after injection. Perfusion of AVP (6.5 μM) within the meA significantly attenuated the hyperthermic response to i.c.v. PGE_1 compared to similar perfusion of aCSF (aCSF = 1.37 ± 0.25 °C, AVP = 0.67 ± 0.15 °C; $p < 0.05$). Perfusion of AVP within the VSA resulted in a more significant suppression of PGE_1 hyperthermia compared to similar perfusion of aCSF (aCSF = 1.34 ± 0.16 °C, AVP = 0.32 ± 0.25 °C; $p < 0.01$). These results suggest that AVP perfused within the meA or VSA suppresses PGE_1 hyperthermia in the urethane-anaesthetized rat. However, AVP perfused within the meA suppresses PGE_1 hyperthermia less efficaciously than AVP perfused within the VSA.

496.9

THERMOREGULATORY CONSEQUENCES OF HIPPOCAMPAL TRANSECTIONS AND SEPTAL LESIONS IN HAMSTERS: DELAYED RESPONSES TO EXOGENOUS PYROGEN AND DELAYED DROP IN NOCTURNAL TEMPERATURE. P.Johnson and K.T.Borer, Dept. Kinesiology, Univ.of Michigan, Ann Arbor, MI 48109.

To characterize the thermoregulatory consequences of rostromedial septal lesions (SEP) and hippocampal transections (HIPPO), mature female golden hamsters were implanted with temperature telemetry devices for monitoring of core temperature (t_c, Minimitter Co.) one week prior to SEP (n=8), HIPPO (n=6), and control (CON, n=6) neurosurgery. On days 10,12, and 14 postsurgery, responsiveness of lesioned hamsters to the lipopolysaccharide (LPS) pyrogen was checked by injecting each animal ip with saline or two doses of LPS (10 and 20 ug/kg). Lesions resulted in: (1) a 5h (SEP) to 9h (HIPPO) delay in the decline of nocturnal t_c, and in response to injected LPS, (2) a reduced early phase in t_c rise, and (3) a 3 to 5 h delay in the second phase of t_c rise. Hippocampus, and to a lesser extent the rostromedial septum in hamsters play a role in nocturnal tc decline and in pyrogenic response to LPS.

496.10

EFFECT OF INTERLEUKIN-1α ON RADIATION-INDUCED HYPERTHERMIA IN RATS. S. B. Kandasamy, K. S. Kumar*, A. H. Harris*, and J. F. Weiss*. Behavioral Sciences and Radiation Biochemistry Departments, Armed Forces Radiobiology Research Institute, Bethesda, MD 20814-5145.

Exposure of rats to 10 Gy of high-energy electrons (18.6 MeV) produces hyperthermia and decreased hypothalamic levels of the antioxidant enzymes superoxide dismutase (SOD) and glutathione peroxidase. Intracerebroventricular administration of 1-5 μg of SOD or 1-5 units of glutathione peroxidase attenuated radiation-induced hyperthermia. Recombinant human interleukin-1α (IL-1α) administered 24-hr before radiation exposure increased the levels of antioxidant enzymes and prostaglandin E_2 (PGE_2) and reduced the hyperthermic response. These effects were blocked by the cyclooxygenase inhibitor indomethacin and also did not occur when IL-1α was administered 1-hr before radiation exposure. The results suggest that the attenuation of radiation-induced hyperthermia by IL-1α may involve PGE_2 synthesis and the stimulation of antioxidant enzymes.

496.11

RESPONSES OF PREOPTIC NEURONS TO HUMAN RECOMBINANT INTERLEUKIN-6 (IL6) IN TISSUE SLICES. L. Xin* and C.M. Blatteis. Univ. of Tennessee, Memphis, TN 38163.

It has been suggested that IL6 may be another endogenous pyrogen (EP) because, like other such agents, its i.v. injection induces monophasic fevers, albeit at much higher doses than the others. We recently showed that its site of action in guinea pigs is the preoptic area (PO); this is also the locus of the pyrogenic action of the other EPs. This brain region contains cold- (C) and warm- (W) sensitive neurons that generally are excited and depressed, respectively, during the febrile rises other EPs induce, while thermally insensitive neurons are affected unpredictably. This study was undertaken to determine whether the neuronal action of IL6 is analogous to that of the other EPs. Extracellular single-unit activities of warm-sensitive and thermoinsensitive neurons were recorded in 350 μm-thick slices of guinea pig PO perfused with oxygenated artificial cerebrospinal fluid. Thermosensitivity was assessed by firing rate changes over the perfusate temperature range 32-42°C; no C units were identified. IL6, added directly to the perfusate at 1 μg/10 μl, depressed the activities of all the W units recorded. The decrease in firing rate was (means ± SD) 2.9 ± 2.2 imp/s, with an onset latency of 6.0 ± 1.3 min and a duration of 34.2 ± 17.7 min. These findings indicate that the effects of IL6 on preoptic thermosensitive neurons are similar to those of other putative EPs, but last longer. All the thermoinsensitive neurons in the PO were also affected by IL6, responding with either an increase (67%) or a decrease (33%) in activity. (Supported by NIH NS 22716 and BRSG RR05423).

496.12

FOOD INTAKE AND BODY TEMPERATURE RESPONSES OF RATS TO RECOMBINANT HUMAN INTERLEUKIN 1-B (IL-1B) AND IL-1B ANTAGONIST (IL-1B ANTAG). C.L. McLaughlin, G.J. Rogan and C.A Baile. Animal Sciences Division, Monsanto Company, St. Louis, Mo 63198.

The cytokine Il-1B, produced by activated macrophages, may mediate the cachexia associated with cancer. Food intake (FI) is decreased by acute but not chronic administration of IL-1B in rats. Il-1B also induces analgesia and this effect was blocked by Lys-D-Pro-Thr, an IL-1B antag (Ferriera, S.H., et al., Nature 334:698, 1988). In the present experiment the ability of this IL-1B antag to block the FI and body temperature (BT) responses to IL-1B were tested in male Sprague-Dawley rats. IP administration of 1.25, 1.88 and 2.50 μg IL-1B decreased FI 13, 20 and 19% 4-22 hours after treatment (25.2, 21.9, 20.2 and 20.3 g, respectively, $p<.05$). Cumulative 22-hr FI was decreased for the 2 higher doses only. In experiment two the decreased FI intake 4 hr after 1.25 μg IL-1B (3.0 vs 5.5 g, $p<.01$) was blocked by coadministration of 5 mg/rat Il-1B antag (4.0 vs 5.5 g, NS) but not 1 mg or less (300 or 600 μg). However, 22-hr FI was decreased similarly by 1.25 μg IL-1B alone and in combination with 1 or 5 mg IL-1B antag (25.0, 25.0 and 26.0 vs 32.0 g, respectively). The IL-1B antag alone (2.5, 5.0 and 10.0 mg/rat) did not affect cumulative 22-hr FI (31.1, 31.3, 29.5 and 31.6 g, respectively). Increased BT 4 hrs after 1.25 μg/rat IL-1B (.9 C vs control, -1. C, $p<.01$) was blocked by 5 and 25 mg IL-1B (.3 and .2 C, respectively, NS). BT 22 hrs later was not affected. It is concluded that IL-1B antag can block FI and BT responses to IL-1B in addition to the analgesic responses demonstrated previously.

496.13

DISTRIBUTION OF CYCLOOXYGENASE (PGH2 SYNTHASE)-LIKE IMMUNOREACTIVITY IN THE OVINE CENTRAL NERVOUS SYSTEM. C.D.Breder and C.B. Saper. Depts. of Pharm. & Physiol. Sci. and Neurology, Univ. of Chicago, Chicago IL 60637

Cyclooxygenase (COX) is the rate limiting enzyme in the conversion of arachidonic acid to prostaglandins. These lipid mediators have been implicated in numerous central nervous system activities including generation of the febrile response, modulation of peptidergic neurotransmission in the cerebral cortex and depolarization of sensory afferent neurons. We mapped in detail the distribution of COX-like immunoreactive (ir) structures in the ovine central nervous system with two polyclonal antisera raised against the purified enzyme. COX-ir neurons were observed throughout the cerebral cortex in a distinct laminar pattern that varied in different areas. COX-ir neurons were particularly numerous in the basal forebrain, particularly in the tubero- mammillary and dorsomedial hypothalamic nuclei, the basal amygdala and the bed nucleus of the stria terminalis. In the brainstem they were found in somatic sensory cell groups including the dorsal column nuclei and the spinal trigeminal nucleus as well as visceral sensory regions such as the nucleus of the solitary tract and parabrachial nucleus. COX-ir non-neuronal structures were observed in association with the circumventricular organs of the third and fourth ventricle. We propose that COX may be present within the CNS particulary in neuronal pathways involved in sensory and autonomic function.

496.14

EFFECTS OF TEMPORARY LIDOCAINE LESIONS IN THE VSA OF THE BRAIN OF THE ENDOTOXIN-TOLERANT RAT ON BLOOD PRESSURE AND HEART RATE. M.R.H. McCashin,* C. Shaw, and N.W. Kasting, Dept. Physiology, University of British Columbia, Vancouver, B.C. Canada V6T 1W5

There exists a close correlation between blood pressure and body temperature in rats with first time endotoxin fevers. AVP released into the VSA, reduces body temperature during fever. Blood pressure sensitive neurons have been located in the VSA, therefore neuronal connections within the VSA may play an important role in modulation of body temperature by blood pressure during fever in the rat. Telemetry was used to measure MABP and HR from unrestrained rats. Brain and rectal thermistors were used to measure body temperature (Tb). Rats were rendered tolerant by injections of endotoxin every 3 days. The VSA was infused for the length of the experiment with 5% lidocaine or vehicle (1 ml/hr). Linear regression analysis of vehicle-treated tolerant rats showed no relationship between Tb and MABP whereas lidocaine-infused tolerant rats had a good correlation (re=0.74). These results lead us to conclude that the VSA mediates some of the pathways between Tb and MABP in the tolerant rat.

NEURAL-IMMUNE INTERACTIONS II

497.1

PRENATAL EXPOSURE TO DIETHYSTILBESTEROL AND CHANGES IN THE IMMUNE SYSTEM. N.R.S. Hall and M.P. O'Grady. Psychoimmunology Div., Dept. of Psychiatry, Univ. of South Florida College of Med., Tampa, FL 33613.

We have previously shown that thymosin peptides can modulate reproductive neuroendocrine circuits and that activation of the immune system during ontogeny can modulate the adult reproductive axis. In this study, we evaluated the effects of early exposure to a synthetic estrogenic compound, diethylstilbesterol (DES). Pregnant SW mice were injected with 1 μg of DES either between days 11-15 or days 17-20 of gestation. Control mice received the same volume of oil vehicle or remained uninjected. Overall, early exposure to DES was found to enhance mixed lymphocyte responsiveness, and blastogenesis in response to Con-A, PHA and LPS in the 4-6 week old male and female offspring. Control females had increased thymus weight and spleen cell blastogenic responsiveness compared with control males while DES exposure increased the male measures toward those of the untreated female offspring. These results suggest that exposure to estrogenic compounds during fetal development can alter the immunologic status of the adult. Supported in part by a grant from the NIH (NS21210).

497.2

IMMUNOLOGIC DISPARITY IN THE HYPOPITUITARY DWARF MOUSE. RJ Cross; LE Boyarsky and TL Roszman. College of Medicine, University of Kentucky, Lexington, KY 40536-0084.

The DW/J dwarf mouse, a homozygous recessive mutation lacks growth hormone (GH) and prolactin and can be characterized by extremely stunted growth. Reports by several laboratories indicate that these mice are also compromised immunologically implicating a role for neuroendocrine hormones in the development thymus and spleen and are deficient in both humoral and cell-mediated immunity. These immunological abnormalities can be corrected by supplemental treatment with GH and thyroxine or GH alone. However, more recent data show that these mice are not impaired immunologically and have a normal percentage of T-cell and B-cells in the spleen and cell-mediated immunity to oxalazone, a skin sensitizing agent. Observations by this laboratory indicates an immunological disparity exists in this strain of mice. Although all mice are small in size, some have a normal percentage of T-cells, B-cells and T-cell subsets in the spleen, while others show an abnormal predominance of T-cells over B-cells. The thymus is also not normal in that th population of $CD4^+$, mature T-helper cells predominate with very few immature CD4/CD8 cells, the cell type normally found in highest frequency. (Supported in part by USPHS grant NS22512).

497.3

EFFECTS OF THYMOSIN α_1 ON PITUITARY HORMONE RELEASE. L.Milenkovic* and S.M.McCann. Dept. Physiol., UT Southwestern Medical Center, Dallas, Texas 75235-9040.

We have studied the effects of thymosin α_1 ($T\alpha_1$) on TSH, ACTH, Prl and GH release. To evaluate its effect in vivo we injected the peptide into the third ventricle (3V) of conscious male rats, and measured the concentration of the pituitary hormones in plasma at different times after injection. Following 3V injection of $T\alpha_1$, there was a significant, dose-related decrease of plasma TSH and ACTH concentrations on comparison to control groups. Also, a significant decrease of plasma Prl was observed. There were no significant changes in plasma GH. To examine the effect of $T\alpha_1$ at the pituitary level we incubated hemipituitaries in vitro with different concentrations of the peptide. In this system $T\alpha_1$ evoked a dose-dependent release of TSH and ACTH, while there was no effect on the release of Prl and GH. The results indicate a hypothalamic effect of $T\alpha_1$ to decrease release of TSH, ACTH and Prl. Acting directly at the pituitary level, the peptide stimulates release of TSH as well as ACTH. Both hypothalamic and pituitary sites of action might be of importance for the interaction between central nervous, endocrine and immune system.

497.4

CHOLINE O-ACETYLTRANSFERASE (ChAT) ACTIVITY IN THE ADULT MOUSE THYMUS. M. Badamchian, J. Hausman, T. Radojcic* and K. Bulloch Dept. of Biochem, Mol. Bio. George Washington Univ. Washington, D.C. 20037; Dept of Psychiatry Univ. of Cal. San Diego, La Jolla, CA 92093.

Acetylcholinesterase and muscarinic acetylcholine receptors have been identified in the adult mouse thymus. However, specific ChAT has only been identified within thymic nerves via immunocytochemistry. In this study, we have evaluated the thymuses and brains of fourteen adult male and female BALB/c mice, five to six weeks of age for specific ChAT activity as described by Badamchian and Carroll, J. Neurosci.,5:1955,1985. Our results show that ChAT activity for whole brain was 9.9 +/-0.76 µmol/min/mg protein and 2.51 +/-0.72 µmol/min/mg protein for the thymus. Higher levels of ChAT activity would be expected for tissue such as the brain (which includes both cholinergic neuronal cell bodies and fibers) than for thymus (which contains only cholinergic neuronal fibers). Future studies will determine if the activity of this enzyme in the thymus is influenced by development, circadian rhythms or hormonal stimulation. Supported by ONR grant #N00014-89-J-1256.

497.5

LUTEINIZING HORMONE-RELEASING HORMONE GENE EXPRESSION IN THE THYMUS. B. Marchetti, C.C. Maier*, R.D. LeBoeuf*, and J.E. Blalock*. Dept. of Pharmacology, Univ. of Catania, Italy and Dept. of Physiology and Biophysics, Univ. of Alabama at Birmingham, Birmingham, 35294 U.S.A.

The luteinizing hormone-releasing hormone (LHRH) gene is expressed in hypothalamic and extra-hypothalamic brain regions, as well as in mammary gland, gonads, and placenta. These results suggest that LHRH plays an important functional role in the control of reproductive functions. Recent evidence (Marchetti, B. et al., Endocrinology 125:1025, 1989) indicates that LHRH is able to directly influence lymphocyte function both in vivo and in vitro via specific LHRH receptors. In this report, we show that LHRH is expressed by immune cells. Poly A$^+$ mRNA from murine thymocytes and splenocytes was used as a template for selective RNA-PCR. Amplification primers were designed to yield a 330 bp cDNA product. PCR products from thymocyte and splenocyte mRNA preparations using a LHRH-specific ^{32}P-labelled oligodeoxynucleotide probe showed that the coding sequence for LHRH was present. These LHRH cDNAs from lymphocytes will be sequenced and compared to the sequence of hypothalamic LHRH. The results of this study provide the first characterization at the molecular level of LHRH gene expression by cells of the immune system and identifies another potential regulatory circuit between the neuroendocrine and immune systems.

497.6

ACCELERATED DEVELOPMENT OF MONOAMINERGIC INNERVATION OF KIDNEY, SPLEEN AND THYMUS IN SPONTANEOUSLY HYPERTENSIVE RATS (SHR). Vincent H. Gattone II, and Edwin S. Purcell*. Department of Anatomy and Cell Biology, The University of Kansas Medical Center, Kansas City, Kansas.

The SHR has several pathologic traits which accompany the elevated blood pressure, including increased actuivity of renal sympathetic nerves, renal damage and immune deficiency compared to the normotensive Wistar Kyoto rat (WKY). The augmented sympathetic nerve activity is thought to contribute to the pathogenesis of hypertension as well as the vascular abnormalities in SHR. We propose that augmented or early sympathetic innervation might contribute to renal changes and immune deficiency in the SHR. The development of monoaminergic nerves in kidney, spleen and thymus of SHR and WKY rats (newborn, 1, 2, 3 and 12 weeks of age) was examined using the glyoxylic acid histofluorescence method. Coded specimens were examined using epifluorescence microscopy and evaluated for extent and intensity of fluorescence from monoaminergic nerves. The innervation was scored (0-5) for thymus and spleen. Norepinephrine levels were determined for kidney. Development of sympathetic innervation was accelerated in SHR in all three organs. The kidney and thymus exhibited a sustained increase in innervation while the spleen exhibited only a transient increase. In conclusion, kidney, thymus and spleen are innervated precociously in the SHR as compared to the WKY. Renal innervation may predispose the kidney to hypertensive damage while thymic and splenic innervation may contribute to the immune deficiency of the SHR. Supported by PHS grant #MH46511.

497.7

CORTICOSTERONE REGULATION OF ADRENAL STEROID RECEPTOR LEVEL IN BRAIN AND IMMUNE TISSUE. R.L. Spencer, A.H. Miller* and B.S. McEwen. Lab. of Neuroendocrinology, Rockefeller Univ., New York, NY 10021.

We have compared the extent of adrenal steroid (AS) receptor up- and down-regulation that occurs between different tissues in response to removal of endogenous adrenal steroids (via adrenalectomy; ADX) and replacement with exogenous corticosterone (CORT; via subcutaneous pellets). Male S.-D. rats (300-350 g) were ADX and some were given replacement doses of CORT for 5 days producing plasma CORT levels ranging from low to high physiological values. All CORT pellets were removed 24 h before sacrifice and RIA of trunk blood from each individual animal indicated that there was no residual CORT at the time of death. An additional short-term ADX (24 h) group served as the reference for the number of AS receptors present in the non up- or down-regulated state. The type I AS receptor did not up-regulate with 6 days of ADX, but was down-regulated significantly in the hippocampus by both the medium and the high dose of CORT replacement, and in the spleen by the high dose of CORT. The type II AS receptor was significantly up-regulated in all brain areas examined and in the spleen after 6 days of ADX. The high dose of CORT replacement significantly down-regulated type II receptors in a variety of brain areas (hippocampus, cortex, septum/basal forebrain, and cerebellum) and in immune tissues (thymus, spleen, and peripheral blood mononuclear cells), but not in the pituitary. These data indicate that the extent of AS receptor up- and down-regulation varies between different tissues and may have relevance for tissue differences in the functional responsiveness to chronic glucocorticoid treatment. (Supported by MH 41256)

497.8

IMMUNOSUPPRESSION BY CHLORPROMAZINE IS NOT RELATED TO ITS DOPAMINE ANTAGONIST PROPERTIES. H.U. Bryant, P.L. Berry*, R.E. Roudebush*, N.K. Layman*, R.L. Cain* and L.D. Butler*. Dept. Immunology, Eli Lilly and Co., Indianapolis, IN 46285.

A number of neurologically active substances affect the immune system via either direct or indirect mechanisms. For example, immunomodulatory effects have been attributed to the neuroleptic phenothiazine, chlorpromazine (CPZ). CPZ possesses a complex pharmacology; a particular interest to us was its ability to act as a dopamine receptor antagonist. Therefore, a series of experiments were conducted to evaluate the effects of dopaminergic antagonists on a battery of immunologic parameters. Mitogen elicited mouse spleen cell proliferation *in vitro* was inhibited by CPZ, trifluoperazine (TFP), sulpiride (SUL) and haloperidol (HAL; IC_{50} values of 50, 5, 1.4 and 1 µM, respectively). Metoclopramide (METO) had no effect. *In vivo* lymphocyte proliferation in mice (uptake of iododeoxyuridine [IUDR]) was markedly suppressed (85%) by relatively high doses of CPZ (10 to 15 mg/kg) which also produced sedation (inverted screen test). TFP and HAL at 10 mg/kg produced mild reductions of IUDR uptake, while SUL and METO had no effect. A murine graft verses host response was unaffected by HAL, SUL and METO, and was modestly inhibited by TFP (45% at 30 mg/kg) and CPZ (30% at 15 mg/kg). No suppression was observed in a picryl chloride induced delayed type hpersensitivity response. These studies indicate that more specific dopamine antagonists (e.g. SUL and HAL) do not share the immunomodulatory profile of CPZ, suggesting these effects of CPZ are not related to its dopamine antagonistic properties and are due to another activity of the phenothiazine.

497.9

THYMIC OXYTOCIN RECEPTORS: STEROID EFFECTS AND DEVELOPMENTAL CHANGES. Caldwell, J.D., Walker, C.H., Pedersen, C.A., Peterson, G., Noonan, L. R., and Mason, G.A.; Dept. of Psychiatry and BDRC, Univ. of North Carolina, Chapel Hill, NC. 27599-7250

The presence of oxytocin (OXT) immunoreactivity and expression in the thymus gland led to the discovery of OXT receptors in thymocytes. Using the OXT specific analogue ornithine-vasotocin, we studied OXT receptors in membrane fractions from the thymus of neonatal and adult rats. In adult ovariectomized rats given one of four different steroids (5 ug daily for three days) we found several changes in OXT receptors. Testosterone (T) and estradiol (E) both increased OXT receptor affinity (K_D = 0.2 nM for oil, and 0.12 for both E and T) while decreasing receptor densities (B_{max} = 10.1 fmol/mg protein for oil vehicle, vs. 5.5 and 6.4 for E and T). Corticosterone and progesterone (P) reduced receptor densities (B_{max} = 7.5 for both) without affecting receptor affinity. Giving 500 ug P 5 hours before decapitation to animals treated with 5 ug E resulted in an increase in the density of thymic OXT receptors (B_{max} = 6.0 versus 8.8 fmol/mg protein for E only versus E & P). We found thymic OXT receptors in pups as early as day 4 after birth. The density of thymic OXT receptors increased from day 4 to day 22 of development (from B_{max} = 0.266 $\pm$ .08 to 0.621 $\pm$ .05 fmol/mg protein). Oxytocin release in infants may be an important factor in establishing immune competence.

497.10

CALCIUM ENHANCES cAMP PRODUCTION IN T LYMPHOCYTES STIMULATED THROUGH THE ß-ADRENERGIC RECEPTOR. S.L. Carlson and T.L. Roszman*, Dept. of Microbiology and Immunology, Univ. of Kentucky Medical Center, Lexington, KY 40536-0084

Lymphocytes express functional ß-adrenergic receptors that are linked to the cAMP second messenger system. Stimulation of these receptors has been shown to alter lymphocyte activation and function. To understand the mechanism of ß-adrenergic effects on T cells, we have investigated the intracellular signals generated in human T cells in response to stimulation with a ß-adrenergic agonist (isoproterenol, ISO) and a mitogen (phytohemagglutinin, PHA). Dual stimulation of T cells with ISO and PHA results in a synergistic rise in cAMP production (J. Neuroimmunol. 24:155, 1989). PHA alone does not stimulate significant cAMP, but rather signals the cell via the PI cycle resulting in increased intracellular calcium ($[Ca^{2+}]_i$) and activation of protein kinase C.

Increased Ca^{2+} is one possible mechanism by which PHA stimulation could enhance ß-adrenergic-linked cAMP production. To examine the effect of increased Ca^{2+}, two agents were used that increased $[Ca^{2+}]_i$ by different mechanisms. Ionomycin acts at the level of the plasma membrane to carry Ca^{2+} ions across the membrane from the extracellular medium. Thapsigargin, (gift from Dr. Michael Hanley, Univ. Cambridge) acts at the level of the endoplasmic reticulum to inhibit the Ca-ATPase pump, resulting in increased $[Ca^{2+}]_i$. Neither agent had an effect on cAMP levels in T cells when used alone, but when paired with stimulation of the ß-adrenergic receptor caused a synergistic increase in cAMP. These results suggest that the Ca^{2+} fluxes that result from mitogen stimulation of T cells are involved in the synergy of cAMP production in ISO-stimulated T cells. The stimulation of T cells by PHA and ISO in Ca^{2+}-free medium resulted in enhanced cAMP production, presumably because of the release of Ca^{2+} from intracellular stores. Collectively these results indicate that Ca^{2+} has a role in modulating cAMP production in T cells. (Supported by NS-17423 and F32-NS08591)

497.11

FETAL ALCOHOL EXPOSURE (FAE) ALTERS DEVELOPMENT OF GLUCOCORTICOID CYTOSOLIC RECEPTOR (GCCR) NUMBER IN RAT THYMOCYTES. C.M.K. Wong*, B.J. Branch*, L. Nguyen*, F. Chiappelli and A.N. Taylor. Sch. of Dentistry, Lab. of Neuroendocrinol., Br. Res. Inst., & Psychoneuroimmunol. Prog., UCLA, & West L.A. VAMC/Brentwood, Los Angeles, CA 90024.

We have shown that FAE in the last 15 days of gestation (5% w/v ethanol) in Sprague Dawley rats is associated with long-lasting alterations in the regulation of hypothalamo-pituitary-adrenal (HPA) function and of cell-mediated immunity (CMI). In the context of HPA-CMI interactions, we tested FAE outcome on the development of the number of GCCR sites/cell in thymocytes from 16-72 day-old male rats. Preliminary data suggest a "U"-shaped pattern in normal rats (day 16:5540 sites/cell±294 [SEM, n=2]; day 30:3921±859[3]; day 44: 6823 ±481[3]; day 72:9312±754[4]). FAE rat thymocytes had more GCCR than normal rats at earlier (day 16:107%; day 30: 116%; day 44: 110%), but not later ages (day 72:86%). The control pair-feeding procedure also altered GCCR number compared to normals (day 16: 82%; day 30:119%; day 44:86%; day 72: 67%). Our data indicate that the normal development of GCCR in rat thymocytes mirrors that of the proliferative response of thymocytes to concanavalin A (ConA) but that the effects of FAE on thymocyte GCCR number and ConA response development are not perfectly correlated, suggesting that the complex effects of FAE on HPA-CMI circuitry may be mediated only in part by GCCR. (VA Medical Research & Bettingen Fdn.)

497.12

ADRENAL STEROID RECEPTOR ACTIVATION IN VIVO HIGHLY CORRELATES WITH IMMUNE FUNCTION AS MEASURED IN VITRO Miller AH*, Spencer RL, Kim C*, Trestman R*, McEwen BS and Stein M, Mt Sinai School of Med, NY, NY 10029

The relationship between estimates of adrenal steroid (AS) receptor activation in vivo and immune function in vitro was determined in SD rats (n=6) exposed to high (10µg/ml), medium (0.8µg/ml) and low (0.3µg/ml) doses of dexamethasone (DEX) in the drinking water overnight (16 hrs). The following day, spleens were removed for Type I and II AS receptor binding and measurement of Con A induced splenocyte proliferation. DEX administration was associated with a stepwise decrease in Type II AS binding while Type I AS binding was unchanged. In addition, DEX was associated with a stepwise decrease in splenocyte proliferation. A highly significant correlation between Type II binding and the Con A proliferative response was observed (r=.89, p<.0001), indicating that as Type II receptor binding decreases (reflecting receptor activation) so does immune function. No correlation was found between Type I binding and mitogen proliferation. These data support the notion that in order for adrenal steroid hormones to alter immune function, evidence of receptor activation is an important prerequisite. Supported by NIMH MH00680-02 (AHM) and MH46504-01 (AHM).

497.13

NORADRENERGIC INFLUENCES ON NATURAL KILLER CELL ACTIVITY. J. Irwin, L.F. Kuehner* & K.A. Zito. Psychology Dept., Queen's University at Kingston, Ontario, Canada K7L 3N6

Studies of acute stress, as well as pharmacological investigations have implicated norepinephrine (NE) as an agent of immunological modulation. The present study assessed the effects of *in vivo* manipulations of noradrenergic activity on the cytotoxicity of Natural Killer (NK) cells.

Depletions of norepinephrine were induced in male CD-1 mice by administration of 250 mg/kg of alpha-methyl-para-tyrosine (AMPT), an inhibitor of tyrosine hydroxylase. Mice were then sacrificed for determination of NK cytotoxicity against YAC-1 target tumor cells in a 6 hr chromium release assay. There was a reduction in NK activity 3 hr following AMPT administration. However, 24 hr following AMPT administration, NK activity was no longer inhibited. In separate experiments CD-1 mice received I.P. injections of NE or the beta-adrenergic agonist isoproterenol. Surprisingly, NE administration again resulted in a dose-dependent suppression of NK activity. In contrast, administration of the beta agonist enhanced NK cytotoxicity in a dose-dependent fashion. The data suggest that modification of sympathetic nervous system activity, which occurs under conditions of acute stress, may disrupt natural killer cell function.
(Supported by NSERC Grant U0569)

497.14

CENTRAL MU AND NOT KAPPA OPIOID RECEPTORS MEDIATE SUPPRESSION OF NATURAL KILLER CELL ACTIVITY. L.C. Band[1], Agu Pert[2], B.R. de Costa[1*], K.C. Rice[1*], and R.J. Weber[1]. [1]Laboratory of Medicinal Chemistry, NIDDK, NIH and [2]Biological Psychiatry Branch, NIMH, Bethesda, MD, 20892.

Opiates have well-documented immunosuppressive properties. Clinical use of these compounds may promote tumor growth or metastases, or increase the likelihood of postoperative complications. Furthermore, the increased susceptibiltity of opiate addicts to infection suggests that abuse of these agents may carry the additonal risk of depressed immune function. It has been demonstrated that both peripheral and intracranial injection of morphine depresses natural killer cell (NK) proliferation; the periaqueductal grey has been shown to be involved in the mediation of this effect (Weber & Pert, *Science*, 245, 1989). The following studies were conducted in order to determine the opioid receptor subtype mediating opiate-induced immunosuppression. The selective mu agonist [D-Ala^2, NMe-Phe^4, Gly-ol]-Enkephalin (DAGO) and the selective kappa agonist (S,S)-U50,488 were microinjected into the lateral ventricle in Fisher 344N male rats. Three hours after microinjection spleens were removed and assayed for NK activity. DAGO (6-60 nmol) reduced NK activity while (S,S)-U50,488 (20-200 nmol) had no effect. Preliminary data indicate that the high affinity delta receptor agonist [D-Pen^{2,5}]-Enkephalin (DPDPE) does not suppress NK activity. These findings suggest that opiate-induced immunosuppression is mediated primarily through mu-receptors. Further specification of receptors involved in immunosuppression should provide direction for the design of medicinal compounds which do not compromise immune competence.

497.15

FLOW CYTOMETRIC ANALYSIS OF EXCITABILITY TO PROGESTERONE IN PURIFIED HUMAN NATURAL KILLER CELLS. R.N. Mandler, M.D. Domalewski*, L.C. Seamer*, D. Whitlinger*, R. Doshi*, H. Faris* and A.D. Bankhurst*. Departments of Neurology, Rheumatology and Flow Cytometric Lab, The University of New Mexico School of Medicine, Albuquerque, NM 87131.

Natural killer (NK) cell function depends on a myriad of molecules that interact with membrane receptors and ion channels. Steroid-like Na^+ channel agonists such as veratridine and batrachotoxin depolarized human NK cells in a tetrodotoxin (TTX)-sensitive fashion that suggested presence of Na^+ channels (Mandler, R.N., et al., J. Immunol. 144:2365-2370, 1990). This discovery prompted a study of purified human NK cell excitability to a variety of human steroids using flow cytometry and voltage-sensitive dyes. Highly-purified human NK cells (CD16=95 ± 1%) were prepared using a negative panning technique. Progesterone (10-200 uM) depolarized NK cells in a concentration-, time- and temperature-dependent fashion. Neither cortisol, cholesterol, testosterone, 21-HO cortisone, pregnenolone, nor aldosterone modified NK cell excitability. Progesterone but not the other steroids produced marked effects in NK cell cytotoxicity against K562 tumor target cells. Progesterone effects on purified human NK cell excitability and cytotoxic function might represent one of a number of mechanisms of neuroendocrine modulation of the immune response.

497.16

REPEATED INTRACEREBROVENTRICULAR (ICV) INJECTIONS OF CONJUGATED DOPAMINE (DA-G-BSA) PRODUCE PERIPHERAL ANTIBODIES. O. MRABET*, C. MESSIER, N. MONS*, C. DESTRADE and M. GEFFARD. URA CNRS n°339, U. Bordeaux I, 33405 Talence and Immunol. and Pathol. Lab. CJF 88-13 INSERM, U. Bordeaux II, 3300 Bordeaux Cédex, FRANCE.

Repeated ICV injections of an anti-conjugated dopamine antibody (ACDA) appear to produce CNS lesions particularly in the caudate nucleus (Neurosci. Abstr. 13, 501, 1987). We measured the effect of repeated ICV injections of DA-G-BSA on the peripheral production of ACDA. Mice with an ICV cannula were injected once a week for 5 weeks with either DA-G-BSA, the protein carrier BSA or pre-immune immunoglobulins (IgG); blood samples were collected before each injection. ELISA tests revealed a high sera concentration of ACDA after the third (and following) injection of DA-G-BSA (in 8 out of 9 animals) but not after the injection of either BSA or IgG. Booster ICV injection of DA-G-BSA further increased the sera concentration of ACDA. In another experiment, the same quantity of DA-G-BSA previously injected ICV was injected in the tail vein once a week for 5 weeks. ELISA tests showed only moderate sera concentration of ACDA (in 3 out of 6 animals). We found the CNS immune response to DA-g-BSA to be very strong even though the doses of DA-g-BSA injected were very small and that no immunostimulant was given. The comparison of the results obtained by central and peripheral injections suggest that the peripheral response may be mediated by the combined T and B responses while the central response would depend mainly on the B response.

497.17

REJECTION OF MESENCEPHALIC RETINAL XENOGRAFTS IN THE RAT INDUCED BY SYSTEMIC ADMINISTRATION OF RECOMBINANT GAMMA INTERFERON. T. Subramanian, I.F. Pollack and R.D. Lund. Depts of Neurobiology, Anatomy and Cell Science, and Neurosurgery, Univ. of Pittsburgh School of Medicine, Pittsburgh, PA 15261.

Previous studies have shown that well integrated mesencephalic retinal xenografts can be induced to reject by various systemic and local manipulations (Pollack et al., Neurosci. Abs., 15:11, 1989). In each of these rejection paradigms the development of major histocompatibility complex (MHC) antigen expression on cells in and around the graft coincided with the onset of overt rejection. To determine whether induction of MHC antigen expression was in itself a sufficient stimulus to provoke a rejection response, we examined the effect of systemic administration of rat recombinant gamma interferon (a strong inducer of MHC antigen expression) on xenograft viability.

Post-natal day 1 Sprague-Dawley rats received mesencephalic grafts of embryonic day 13 CD-1 mouse retina. Starting at 21 days of age, one half of these animals received 2 x 10^4 units of rat recombinant gamma interferon intraperitoneally for 6 to 9 days while the other half served as litter matched controls. All the rats were sacrificed 30 days after the final injection and 30 µm thick coronal sections were made through the mesencephalon. Adjacent sections were stained with cresyl violet and antibodies against rat MHC class I (OX-18) and class II (OX-6) antigens, microglia (OX-42), astrocytes (GFAP) and lymphocytes (anti-lym).

Each of the animals that received gamma interferon showed a strong rejection response characterized by perivascular cuffing with mononuclear cells in and around the graft, infiltration of the graft and the surrounding host tissue by lymphocytes and MHC class I- and class II- antigen positive cells resembling reactive microglia. The rejection rate in this group was significantly higher than the control group (Fisher's exact test, p=0.002). These results suggest that gamma interferon is a lymphokine mediator of the initiation and/or subsequent maintenance of the rejection response to cross-species transplants. (Supported by CMRF 0793 and EY 05283 grants)

497.18

DIFFERENTIAL AND SEX SPECIFIC EFFECTS OF KAINIC ACID (KA) AND DOMOIC ACID (DA) LESIONS IN THE LATERAL SEPTAL AREA (LSA) ON BODY WEIGHT (BWt) AND THE HUMORAL IMMUNE SYSTEM. L. Wetmore*, J. Burns* and D.M. Nance. Depts. of Pathology and Physiology, Univ. of Manitoba, Winnipeg, MB, Canada, R3E 0W3.

KA lesions in the LSA of female rats increases BWt and decreases the humoral immune response, relative to controls. DA is a more selective and potent kainate agonist than KA. We tested the effects of LSA lesions produced by DA (0.15 µg in 0.5 µl) on the immune response of female and male rats immunized with ovalbumin. Similar to KA, DA produced an increase in BWt gain of female rats, but reduced BWt gain in male rats. DA produced greater cell loss in the LSA of male rats than in females. Unlike KA, DA lesions had no effect on the immune response of female rats nor was the immune response of male rats altered by the lesions. We reexamined the effects of KA (0.375 µg in 0.25 µl) in the LSA on BWt and the immune response of male and female rats. In females, KA lesions produced an increase in BWt gain and reduced humoral immunity. Similar lesions in male rats had no effect on BWt or immune response. To test if the absence of an effect of DA lesions on the immune system of female rats was due the the small lesions noted in the first study, we tested the effects of more concentrated DA (0.15 µg in 0.25 µl) on the immune response of female rats. This dose produced substantial cell loss in the LSA and a large increase in BWt gain, but the immune response of the DA lesioned females was similar to controls. Thus, both KA and DA lesions in the LSA produce an increase in BWt gain of female rats, but only KA lesions reduce humoral immunity. BWt regulation and immune function were unaltered by DA or KA lesions in the LSA of male rats. Supported by Medical Research Council of Canada.

497.19

BETA ENDORPHIN MODULATES CALCIUM CHANNEL FLUX IN HUMAN NEUTROPHILS. D.B. MILLAR*, D.L. MAZOROW* AND John Thomas. NIMH Neuroscience Center at St. Elizabeths, Washington, DC 20032, and Naval Medical Research Institute, Bethesda, MD 20814. We report here that Beta Endorphin (Bend) also significantly influences n-formyl-methionyl-leucyl- phenyalanine (FMLP) stimulated Ca^{++} entry into human neutrophils. Ca^{++} flux was analysed using the Fura-2 fluorescent technique and the equations described by Mazorow and Millar (FASEB J. 4, #4, A1209 (1990)). 10^{-6} M FMLP induces a Ca^{++} flux in neurotrophils comprising a sharp initial rise and fall followed by a broader rise and fall. Experiments with Prostaglandin d2, dibutyrl cyclic AMP and EGTA showed that the second Ca^{++} peak was reduced in amplitude by the first two compounds and obliterated by the last. In 2.5 x 10^{-5} M nifedipine there is also no second peak. We conclude that the second peak is due to influx of Ca^{++} into the cell via a Ca^{++} channel. Neutrophils incubated for 1 1/2 h at 37° with Bend (10^{-12} to 10^{-6} M) show major changes in the shape and height (f_m) of the second peak in a dose and donor dependent manner. In some donors, f_m is reduced by about 50% as compared to control. In others f_m is increased to values close to that of the initial peak (usually the first peak height is ~2x the height of the second peak). Finally, experiments with naloxone, an opioid antagonist, have not yet satisfactorily shown the reactive site for Bend to be an opiate receptor.

498.1

COMPARISON OF SERUM ANTIBODY RESPONSES TO CNS AND SYSTEMICALLY ADMINISTERED OVALBUMIN (OVA). L.B.Gordon*, M.Kahn*, H.F.Cserr and P.M.Knopf. Physiology and Biophysics, Brown Univ., Providence, RI 02912.

Mechanisms underlying the immunologic privilege of the CNS are poorly understood. We have compared serum antibody responses to OVA, administered either into CNS or systemically in Sprague-Dawley rats, to determine if reduced activity of the afferent limb of the humoral immune response contributes to this phenomenon. 90 μg of OVA was infused either into: the caudate nuclei or lateral ventricle CSF, through cannulas implanted 7 days previously; the nasal mucosa; or the hind footpads. Caudate nuclei-injected animals were challenged 6 weeks later with systemic OVA plus Freund's complete adjuvant. Serum titers of anti-OVA were measured weekly using an ELISA for up to 14 weeks. The fraction of rats with detectable serum titers was 4/9 for caudate nuclei injections (range of all titers: <50 - 2,000), 4/4 for CSF (200-1,500), 0/9 for nasal mucosa (<50), and 0/3 for hind footpads (<50). Caudate nuclei-injected animals with undetectable titers (N=4) responded more rapidly and with higher titers than naive controls to systemic challenge, indicating the presence of memory cells. Results indicate that the afferent limb of the humoral immune response to intracerebral OVA is as active, or more active, than to systemic antigen. Supported by NIH grant NS-11050.

498.2

LYMPHOCYTIC CHORIOMENINGITIS VIRUS (LCMV)-INFUSED RATS EXHIBIT ELEVATED CEREBROSPINAL FLUID (CSF) IMMUNOGLOBULIN A (IgA). A.H.Woo, P.M.Knopf, and H.F.Cserr. Program in Molec. Bio., Cell Bio., and Biochem. and Section of Physio. and Biophys., Brown Univ., Providence RI 02912.

We have developed a nonpathologic, nontraumatic rat model in which to study the humoral immune reponse to virus in the central nervous system (CNS). Fisher rats were microinfused with UV-inactivated LCMV through a lateral ventricle cannula implanted 7 days previously. At 21 days, CSF and serum immunoglobulin (Ig) titers and concentrations of IgA and albumin were determined by enzyme-linked immunosorbent assays. Rats infused with cell culture supernatant or saline and sham-operated rats were used as negative controls. An Ig titer index and IgA index were used to evaluate total CSF Ig and IgA with respect to blood-brain barrier (BBB) integrity where Index = [CSF Ig titer or IgA/serum Ig titer or IgA] / [CSF Alb/serum Alb]. Of 6 LCMV-infused rats, 3 exhibited an elevated total Ig titer index and 2 exhibited an elevated IgA index (> log mean + 2 SD of controls, n=11). We are investigating whether this specific appearance of IgA within an intact BBB is due to a secretory transport mechanism analogous to that found in mucosal tissues outside the CNS.

Supported by NS-11050.

498.3

STUDIES ON THE FUNCTION OF N-ACETYL MURAMYL DIPEPTIDE IN THE INDUCTION OF AUTOIMMUNE DISEASE. F.C.Westall and Ali Mohammadi. Institute for Disease Research, P.O.Box 1293, Alta Loma, CA and California State Polytechnic University, Pomona, CA 91768.

Acetyl muramyl dipeptide (MDP) derived from gut bacteria, is found throughout the human body. As an adjuvant, it has been used to induce autoimmune diseases, eg. experimental allergic encephalomyelitis (EAE) and artifical castration. For induction of either disease, the association of MDP with the autoantigen is quite important. We have examined whether MDP functions by suppressing the proteolytic digestion of the antigen. If potential antigens are digested too rapidly they would not be available for immunoprocessing. MDP does suppress the digestion of these antigens. However, since both antigens themselves show remarkable stability against the proteases utilized, it would seem unlikely that this is a function of MDP. More probably the MDP-complex is immunoprocessed as a unit. Finally it would appear that digestibility of potential immunogens plays a more important part in determining what is immunogenic.

498.4

PRAZOSIN TREATMENT ATTENUATES MONOAMINERGIC AXONAL DAMAGE DURING DEVELOPMENT OF EAE IN RATS. S.R.White, G.Samathanam*, P.Black* and K.Owada*. Dept. of VCAPP, Washington State Univ., Pullman, WA 99164.

Our laboratory has found that extensive damage occurs to descending monoamine- and peptide-containing axons during development of the CNS autoimmune disease experimental allergic encephalomyelitis (EAE). This damage is usually found near perivascular and submeningeal inflammatory foci. The α-adrenergic antagonist, prazosin, suppresses the clinical signs of EAE and delays the inflammatory response in the spinal cord (Brosnan et al., Proc. Natl. Acad. Sci. 82:5915, 1985). The present study used immunohistochemical techniques to determine whether prazosin also prevents axonal damage in rats that have been inoculated for EAE.

Lewis rats were inoculated for EAE with Lewis rat spinal cord homogenate. Prazosin injections began on day 7 postinoculation (2 mg, i.p., twice per day) and continued until sacrifice day (day 15). The control group received equivalent injections of the saline vehicle. The prazosin treatment markedly attenuated clinical signs (hindlimb ataxia was observed in only 1/7 rats, whereas 6/7 control rats developed severe to complete hindlimb paralysis). Inflammation in the spinal cord was attenuated, but not prevented, by prazosin (17.3 ± 1.7 versus 30.2 ± 0.6 foci per 6 mm long section). Prazosin also attenuated, but did not prevent, axonal damage (2.6 ± 0.2 versus 6.8 ± 0.6 5HT axonal damage foci per 6 mm long section). The close correlation between degree of inflammation and severity of axonal damage provides additional evidence that these axons become damaged as they encounter inflammatory foci in the spinal cord.

498.5

ANTIGEN PRESENTATION BY GLIAL CELLS FROM EAE RESISTANT AND SUSCEPTIBLE STRAIN OF MICE.
G. Birnbaum, L. Albrecht* and G. Eichhorn*. Dept. of Neurology, Univ. of Minnesota Sch. of Med., Minneapolis, MN 55455

We studied the antigen presenting properties of glial cells derived from strains of mice resistant (B10.S) or susceptible (SJL/J) to experimental allergic encephalomyelitis (EAE). Indicators were T cell lines sensitized to myelin basic protein (MBP) that were Class II identical with the glial cells.

Monolayers of glial cells from newborn murine cerebral hemispheres were exposed to a mixture of lymphokines from activated T cells to induce Class II MHC expression on glial surfaces. Glia were irradiated and varying amounts of MBP (0.25, 2.5, or 25 μg/ml) were placed onto the cultures along with 1 X 10^5 MPB sensitized T cells. After 48 hours the proliferative responses of the T cells were measured using a ^{3}H-thymidine incorporation assay.

At high antigen concentrations (25 μg/ml) B10.S and SJL/J glia functioned equivalently in inducing MBP sensitized T cells to proliferate. At lower antigen concentrations (2.5 μg/ml) glia derived from the EAE susceptible strain, SJL/J, were more efficient at presenting antigen as measured by an increased ability to induce T cell proliferation (multiplicities of 2.9±1.1 for SJL glia vs.1.7±1.1 for B10.S glia). Spleen cells from both mouse strains were identical in their abilities to present MBP indicating differences in presentation were tissue specific.

One factor in determining susceptibility to an organ specific autoimmune disease may be the antigen presenting capabilities of the target organ, in this case the brain.

Supported by the National Institutes of Health

498.6

IMMUNE RESPONSE TO CSF-INFUSED MYELIN BASIC PROTEIN (MBP) IN THE LEWIS RAT. C.J.Harling-Berg, P.M.Knopf, H.F.Cserr. Physiology and Biophysics, Brown Univ., Providence RI 02912.

Our previous work showed that CSF-infused albumin induced a significant systemic antibody response in the Sprague-Dawley rat. Present studies are aimed at evaluating the immune response to a CNS antigen, guinea pig MBP, infused into the CSF of the Lewis rat, a strain susceptible to the CNS demyelinating disease experimental allergic encephalomyelitis (EAE). The immune response was evaluated by studying serum antibodies and EAE expression. As a positive control, the immune response to CSF-infused chick ovalbumin (COA) was studied in a separate group. Antigen (90 or 900μg) in saline (10 or 20μl) was infused (0.5μl/min) through a cannula implanted into a cerebral ventricle. Serum antibodies titers were measured using an ELISA, 14-16 days post CSF-infusion. Rats receiving MBP were scored (0-4) for clinical signs of EAE. Serum anti-COA antibodies were detected in most rats (8/11) and titers ranged from 20 to 2,000. In contrast, serum anti-MBP antibodies were detected in only a few rats (3/15) and these were low (titer 20). Preliminary studies indicate that rats (N=3) receiving a CSF-infusion of MBP prior to an EAE-inducing challenge demonstrate a delay in EAE onset (Day 12 v 16) and less severe symptoms (score 1 v 3) compared to naive rats (N=7). Supported by Natl. MS Soc. and NIH NS 11050.

498.7

IMMUNOLOGIC CHANGES ASSOCIATED WITH SPINAL CORD TRANSECTION IN RATS. J.B. Gelderd^, N.R.S. Hall†, R.A. Menzies*†, M.P. O'Grady†, M. Wiranowska*• and S. Filteau*†. Dept. of Anatomy^, Texas A&M Univ., College Station, TX 77843-1114, and Depts. of Neurology• & Psychiatry†, Div. of Psychoimmunology, Univ. of South Florida College of Med., Tampa, FL 33613.

The study was designed to determine whether reactive astrocytes in the spinal cord are capable of secreting monokines and lymphokines. Male, Sprague Dawley rats ranging in age from 40 to 50 days were anesthetized and a dorsal midline incision was made in the mid-thoracic region. A laminectomy was performed at the T5-T6 vertebral level, and the spinal cord was transected with a scalpel. Sham animals were subjected to the same surgery, but omitting the transection step. A group of unhandled control rats was also included. Four 2mm sections of spinal cord were dissected from each rat at either 24 hours, 72 hours, 7 days or 14 days. The spinal cord segments included adjacent and distal sections both rostral and caudal to the site of transection. Detectable levels of interferon alpha have not been found in these sections at either 24 or 72 hours, although more sensitive assays of interferon activity are currently being assessed along with the later time points. Decreases in thymus and spleen weight were detected at both the 24 and 72 hour time points and these are being correlated with lymphocyte blastogenesis and cytokine production. Since cytokines act upon and are secreted by astrocytes, we are hypothesizing that they are produced and play a physiological role within the neuropil adjacent to the spinal injury. These studies were supported in part by a grant from the NIH, DA05723.

498.8

BRAIN REACTIVE AUTOANTIBODIES IN THE SERA OF RA PATIENTS. C.Madsen* and S.A.Hoffman. Dept. of Microbiology, ASU, Tempe, AZ 85287.

Recent studies have focused on the effects of psychosocial influences on the immune system in chronic immunologic diseases. Relatively little attention has been paid, however, to the potential influence of altered immune system functioning on psychological profiles. Autoantibodies to brain have been demonstrated in patients with various autoimmune diseases. It has been hypothesized that such brain reactive autoantibodies play a role in mediating some of the CNS manifestations seen in these diseases. Rheumatoid arthritis (RA) is an autoimmune disease in which patients exhibit profound depression, although other psychological involvement or CNS manifestations are rarely reported. This study reports the occurence of autoantibodies in the sera of RA patients against integral brain membrane proteins isolated from normal mouse brain and localized regions of human brain. Membrane antigens were extracted by phase separation with Triton X-114. They were separated by SDS-PAGE, blotted onto nitrocellulose membrane and probed with RA sera. Numerous antoantibodies were found to react against whole mouse brain. A smaller number of antibodies were also found to react to human brain. Implications of these findings for the significance of these antibodies with respect to stress and immunity, their genesis, and relation to neuropsychiatric manifestations will be discussed.

498.9

SYMPATHECTOMY OF LYMPH NODES EXACERBATES THE EXPRESSION OF EXPERIMENTAL ARTHRITIS. D. Lorton, D.L. Bellinger, M Duclos, S.Y. Felten, and D.L. Felten. Dept. of Neurobiol. and Anat., Univ. of Rochester Sch. of Med., Rochester, NY 14642.

Adjuvant arthritis (AA) was examined in adult male Lewis rats following local sympathetomy (SYMP) of draining lymph nodes (LN). Rats received bilateral local LN injections of 6-hydroxydopamine (6-OHDA) or vehicle (VEH), then half of the rats from 6-OHDA and VEH groups received injection of Freund's complete adjuvant (FCA) at the base of the tail. The remaining rats from each group received the same volume of mineral oil, resulting in four treatment groups: 6-OHDA/FCA,VEH/FCA, 6-OHDA/oil and VEH/oil. Following these treatments, AA was assessed by changes in body weight, dorsoplantar swelling, arthrogram scoring and radiographic analysis for day (D) 27. SYMP of draining LNs accelerated the onset and exacerbated the inflammation and osteopathic changes associated with AA. Body weights did not differ significantly in any treatment group. Limbs of rats who received VEH/FCA demonstrated bilateral inflammation at D17 post-FCA injection; bilateral inflammation of limbs of rats from the 6-OHDAA/FCA group was apparent by D15 following FCA injection. Dorsoplantar swelling AA groups was increased significantly by D17 compared to the 6-OHDA/VEH groups. Significant differences in dorsoplantar swelling in rats who received VEH/oil compared with either AA group was not observed until D23. At D27, a significant increase in dorsoplantar width was observed for the VEH/FCA group compared to the 6-OHDA/FCA group and both control groups. Analysis of X-rays taken of ankle joints on D27, revealed destructive joint changes in both AA groups compared to both control groups. As was observed with dorsoplantar width at D27 , more extensive joint damage was apparent in analysis in 6-OHDA treated arthritic rats compared to VEH treated AA rats. These finding suggest that the noradrenergic (NA) innervation of draining LN play a regulatory role in processing of a relevant antigen for the initiation of AA changes, and that absence of these NA fibers results in exacerbation of the severity and acceleration of the time of onset of AA.

498.10

SUBSTANCE P (SP) DENERVATION OF LYMPH NODES ATTENUATES THE EXPRESSION OF ADJUVANT INDUCED ARTHRITIS. D.L. Felten, D. Lorton, D.L. Bellinger, M. Duclos and S.Y. Felten. Dept. of Neurobiol. and Anat., Univ. of Rochester Sch. of Med., Rochester, NY 14642.

Adjuvant arthritis (AA) was examined in adult male Lewis rats following local SP denervation of draining lymph nodes (LN). AA was induced by intradermal injection of Freund's complete adjuvant (FCA) in mineral oil into the subplantar area of the right hind paw. One day after injection of FCA or mineral oil, half of the rats from FCA group and mineral oil groups received injections of capsaicin (CAPS) into the right draining LN. The remaining rats from each group were administered the same volume of vehicle, resulting in four treatment groups: FCA/CAPS, FCA/vehicle, oil/CAPS and oil/vehicle. Following these treatments, arthritis was assessed by changes in body weight and dorsoplantar swelling for 20 days. Body weights of AA rats, were significantly decreased by 14 days following FCA injection compared to non-AA rats; however, AA rats did maintain body weight from day 1 of the experiment through the expression of AA 20 days later. Bilateral inflammation of hindlimbs was apparent by day 20 post-footpad injection in the FCA/vehicle group. No inflammation was observed in any limb in non-FCA treated rats. No significant difference in dorsoplantar widths of any treatment group was observed compared to controls from day 0 to day 14. Right dorsoplantar width of AA rats were significantly increased compared to both control groups by day 20. At day 20, a significant increase in left dorsoplantar width was observed only in nondenervated, arthritic group (FCA/vehicle) compared to either control group. At day 20, left dorsoplantar widths of the FCA/CAPS group did not differ from either control group. Further studies are required to determine if this difference represents a delayed onset of AA contralateral to the devervated side or an inability of these limbs to develop the inflammation characteristic of arthritis. These findings indicate a dual role of SP innervation in AA through modulation of both inflammation in the joint, and immune responses in lymphoid organs.

498.11

AGE-RELATED ALTERATIONS IN NOREPINEPHRINE UPTAKE IN THE RAT SPLEEN. D.L. Bellinger, D. Lorton, S.Y. Felten, and D.L. Felten. Department of Neurobiology & Anatomy, University of Rochester School of Medicine, Rochester, NY 14642.

Sympathetic noradrenergic (NA) innervation of the spleen is reduced significantly in aged Fischer 344 rats compared with young adult rats (S.Y. Felten et al., Neurobiol. Aging 8: 159-165, 1987). Norepinephrine (NE) concentration and the number of noradrenergic (NA) terminals in spleen decline by greater than 50% and 80%, respectively, with age. In the present study, we examined the high-affinity uptake of NE in spleens from 3 and 21 month old F344 rats using an *in vitro* spleen slice model coupled with morphometric analysis of NA nerve terminal using fluorescence histochemistry for catecholamines. In spleen slices from 3 and 21 month old rats, uptake of ^{3}H-NE from the external incubation medium into nerve terminals was saturable, sodium-dependent, blocked (approximately 62% inhibition) by μM concentrations of desipramine (a competitive NA uptake inhibitor), and followed Michaelis-Menten kinetics, with an apparent Km of 1-2 μM, characteristic of NE high-affinity uptake in other systems. In spleen slices from 21 month old rats, a 70% decrease in total uptake of NE was observed compared with 3 month old rats. Morphometric analysis confirmed the loss of NA nerve terminals in alternate slice of spleens from 21 month old rats. Estimates of ^{3}H-NE uptake per terminal in 21 month old animals demonstrated an approximate increase in uptake of 70% compared with young adult controls. These findings demonstrate that the loss of NA nerve fibers in spleens from aged rats is accompanied by a significant decline in the total uptake of NE. Further, the efficiency of uptake per NA terminal is enhanced in spleens from aged rats, suggesting an increase in the density of high-affinity uptake binding sites for NE in NA nerve terminals, probably as a compensatory mechanism resulting from the decline in NE concentration and/or loss of NA nerve terminals that occur with aging.

498.12

TUMOR NECROSIS FACTOR alpha AND INTERLEUKIN-1 alpha INCREASE PAIN THRESHOLDS IN THE RAT

A.E.Panerai,P.Sacerdote, P.Ricciardi, M.Bianchi*
Dept.Pharmacology, Schoolof Medicine, Univ. Milano, Milano Italy

Recent evidences show that TNF and IL-1 synthesis is not confined only to cells of the immune system, but it is present also in brain. We investigated whether TNF was able to modify pain thresholds in the rat and since TNF induces IL1 production, we considered this cytokine also. In the hot plate test intracerebroventricolar(icv) injection of TNF at the dose of 1 and 2.5 ng /rat induces a significant increase in pain thresholds at 3 and 5 min: higher and lower doses are ineffective. 5 and 10 ng of icv IL-1 also increase pain thresholds with a time course similar to TNF. The administration of a m-Ab against IL-1 blocks TNF induced analgesia suggesting the involvement of IL-1 in the TNF effect. Anti sera against the endogenous opioids(B-endorphin, dynorfin met-enk) are ineffective on both cytokines. From receptor binding studies it appears that TNF does not bind the opioid receptor. The cyclooxygenase inhibitor indomethacin does not block the effect of either cytokines.
Our data are a further example of the links existing between the nervous and immune system.

498.13

PLASMA β-ENDORPHIN (β-E) AND NATURAL KILLER (NK) CELLS IN CONGENITAL INDIFFERENCE TO PAIN (CIP). R. Bernardini, G. Mauceri*, A. Tine', M.C. Nazzarino*, G. Malaponte*, A. Nicosia* and L. Pavone'. Inst. of Pharmacology, 'Pediatric Clinic, and *General Pathology, Univ. of Catania Sch. Med., I-95125 Catania, Italy.

CIP is a rare inherited syndrome characterized by unresponsiveness to painful stimuli. We have evaluated plasma β-E levels and immune function in two patients affected by CIP. Peripheral blood lymphocytes (PBL) were incubated with graded concentrations of phytoemoagglutinin (PHA), with or without addition of β-E. Incorporation of ^{3}H-thymidine was measured 72 h later. We have also studied NK membrane phenotype expression, by incubating PBL with specific anti-Leu 7 and 9 fluorescent monoclonal antibodies. Finally, we have measured plasma levels of β-E, ACTH, and cortisol. PBL proliferation rate was normal, either after incubation with PHA, β-E alone, or in association. Among the surface antigens studied, NK Leu 7 of patients was decreased significantly ($p<0.01$, t test). Plasma β-E levels of patients were higher ($p<0.01$, t test) than controls. No change occurred in plasma ACTH and cortisol levels.

Results indicate that in CIP high plasma β-E levels are associated to NK cell decrease.

498.14

INHIBITION OF SYMPATHETIC OUTPUT ATTENUATES SHOCK-INDUCED SUPPRESSION OF MITOGENIC ACTIVITY. J. E. Cunnick, D. T. Lysle, M. O. Fraser, M. A. Pezzone, and B. S. Rabin. Center for Brain, Behavior, and Immunity, Dept. of Pathology, University of Pittsburgh School of Medicine, Pittsburgh, PA 15213-2582.

Our laboratory has previously demonstrated that the presentation of 16 electric foot-shocks (5 sec, 1.6 mAmp) to male, Lewis rats can induce suppression of the mitogenic response of whole blood and whole spleen lymphocytes to concanavalin A and phytohemagglutinin. The suppression of the splenic mitogen response can be attenuated by the IP administration of ß-adrenergic receptor antagonists, propranolol and nadolol. These drugs do not attenuate the suppression of the blood mitogen response.

Stimulation of the α2-receptors on sympathetic neurons is known to decrease the release of norepinephrine (NE) from the nerve terminals. Our current study examined the ability of α2-receptor agonists to attenuate the shock-induced immune suppression. The α2-receptor agonist, clonidine, but not midodrine is able to cross the blood brain barrier. We administered the α2-agonists or vehicle, IP, 30 min prior to a stress session. The administration of each drug was able to attenuate the shock-induced suppression of the splenic mitogen response to a significant degree at the highest doses administered (50 & 100 μg/kg clonidine and 5 & 10 mg/kg midodrine). However, neither drug attenuated the shock-induced suppression of the blood mitogen response.

This data supports our earlier finding of two distinct mechanisms mediating the suppression of blood and splenic mitogenic responses. Furthermore, modulation of NE release can significantly attenuate the suppressive effects of stress on splenic, but not blood, mitogenic responses. This data indicates that the peripheral release of NE, in vivo, is responsible for the suppression of the spleen mitogenic response.

NEURAL-IMMUNE INTERACTIONS: INTERLEUKINS

499.1

NOREPINEPHRINE AND PROSTAGLANDIN E2: EFFECTS ON SINGLE UNIT ACTIVITY RECORDED FROM THE RAT HYPOTHALAMUS IN VIVO. W. McVaugh and N. Dafny. Dept. Neurobiology and Anatomy, University Texas Medical School at Houston, TX 77225.

Both prostaglandins and activation of the central noradrenergic system have been associated with IL-1's induction of corticotropin releasing factor (CRF) from the PVN into the portal circulation. The goal of this study is to examine the effects of locally applied norepinephrine (NE) and prostaglandin E2 (PGE2) on the electrical activity of single neurons recorded from the anterior hypothalamus/paraventricular area in the anesthetized rat. Male Sprague Dawley rats (280-320g) were anesthetized with urethane (1.2g/kg bw ip) and placed in a stereotaxic instrument. The skull was bared, a 5mm hole drilled in the cranium and the dura reflected. Norepinephrine and PGE2 were iontophoresed onto individual neurons by means of a multibarrel micropipette assembly with a recording barrel attached. Preliminary results indicate that the percentage of neurons responding to NE with a change from baseline activity increased with dose (20,40,80nA - 41,59,65% respectively) and that all doses resulted in excitation. Little change was seen in the overall number of cells responding to 3 doses of PGE2 (20,40 and 80nA - 50,44 and 54% respectively). The two lower doses resulted primarily in excitation while the highest dose produced inhibition in the majority of responders. Previously we demonstrated that IL-1 itself was capable of exciting these neurons as well. These results suggest that: 1) IL-1's activation of the NE input to the PVN may stimulate CRF release and 2) PGE2 induced by IL-1 may also stimulate PVN CRF neurons.

499.2

Cytokine-Associated Axonal Changes Coincide Temporally With Circulating Tumor Necrosis Factor After RIL-2 Infusion. M.D. Ellison* and C.W. Christman, Dept. of Anatomy, Medical College of Virginia, Richmond, VA 23298

In brains of rIL-2-infused rats, we have recently reported alterations of axonal ultrastructure reminiscent of those described in murine spinal cord slices treated with tumor necrosis factor (TNF). These axonal changes were accompanied by other neuronal and glial abnormalities similar to those previously reported in various inflammatory demyelinating conditions and recently linked, by other investigators, to cytokines. In our laboratory, such alterations were seen as early as 4 hours after a single rIL-2 infusion and were also observed after 3 and 5 days of rIL-2 infusions, administered 3 times daily. The present study was undertaken to determine whether rIL-2-related brain parenchymal alterations in rats are accompanied temporally by elevations of circulating TNF. Adult male rats were infused once (N=6) or thrice daily for 3 days (N=7) with rIL-2, IV (6×10^5 IU/kg, Cetus Corp.). Controls (N=15) were comparably infused with rIL-2 vehicle. Venous blood samples were drawn prior to infusion in 16 additional animals and at 2,4, and 8 hours after the final infusion in rIL-2/vehicle recipients. Serum TNF activity was determined by L929 fibroblast bioassay (kindly performed by Cetus Corp.). In 14 of 16 samples drawn prior to infusion and in all serum samples from animals infused with rIL-2 vehicle, TNF acfivity was below the assay level of detection. Serum samples from rats infused once with rIL-2 ranged in TNF activity from 34 to 430 ng/ml. Animals receiving rIL-2 over 3 days exhibited serum TNF activity ranging across animals from 0.31 to 56 ng/ml, except for 1 8-hr. sample (430 ng/ml). TNF levels in the multiple rIL-2 infusion group were statistically lower than those measured in the single infusion group. Although TNF activities varied across animals in each group, activities for each animal generally remained constant across sample times. We concluded from these data that, after systemic rIL-2 administration, elevations of circulating TNF are temporally coincident with the presence, in brain, of axonal changes similar to those previously associated with TNF, in vitro. Whether circulating TNF and cytokine-linked morphological CNS abnormalities are causally related remains to be determined and merits further investigation. (Supported by NS 25871).

499.3

DIFFERENTIAL STAINING FOR NEUROIMMUNE SUBSTANCES IN BRAINS OF ETHANOL (ETOH) ADMINISTERED RATS. I. Sterzl and G.P. Kozlowski. Department of Physiology, University of Texas Southwestern Medical Center, Dallas, TX 75235-9040.

The ABC Elite kit (Vector Labs) was used to test if ETOH may alter immunoreactive CNS antigens to six antisera: rab & sheep anti-rat IgG, rab anti-rat albumin (Arnel Inc), mAb 11B11 rat anti-mouse Il-4 (Ohara & Paul) & CD4 (Vitetta GK1.5), and mAb1403 mouse anti-rat homologue of human CD4 (Chemicon Int). As previously described (Neurosci Abstr 15,416), male rats were simultaneously pair-fed either an ETOH or control liquid diet for 30 days or Lab Chow and water *ad lib*. There was considerable hypothalamic and hippocampal staining for IgG, Il-4, and CD4. MAb GK1.5 stained equivalent structures to mAb1403 but was less immunoreactive. In general, the ETOH animals had more neurons stained for IgG, Il-4 and CD4 but not albumin when compared to controls. The ETOH animals had twice the IgG-containing neurons in the arcuate nuc and subfornical organ but the staining was diffuse rather than punctate as found for all other areas and antisera. In controls, Il-4 and CD4 staining was greater in supraoptic nuc (SON) than paraventricular nuc (PVN) but ETOH treatment reversed this pattern by enhancing PVN and reducing SON staining. Supported by AA-06014.

499.4

IMMUNE SYSTEM ACTIVATION INCREASES GFAP ICC IN HYPOTHALAMUS. X. Ou, M.N. Gordon and D.G. Morgan. Andrus Gerontology Center, U Southern Cal, L.A., CA 90089-0191

This study is a preliminary report of our attempts to determine the nature of immune system modulation of CNS function. Normal immune activity is regulated by multicomponent feedback loops between the immune system and the nervous system. IL-1 is suggested to be one substance that conveys information from the immune system to nervous system. Our initial attempt was to measure the IL-1 level in CNS following immune system activation using immunohistochemical procedures, but this has not been successful due to lack of antibodies reacting with the rat IL-1 variant. Since IL-1 has been reported to activate astrocytes, we measured GFAP levels immunohistochemically as a potential indicator of IL-1 activity in CNS. In this study, male Sprague Dawley rats were injected with 10% sheep red blood cells in physiological saline (4ml/kg body weight, i.p.) to activate the immune system. Control rats were similarly injected but with saline. Vibratome sections were prepared from animals killed 2 hours, 1 day and 7 days post-injection, and stained immunohistochemically for GFAP. There was no significant change in hypothalamic GFAP staining 2 hours after the injection. However, at both 1 and 7 days after the rbc injections there were increases in GFAP staining (about 3-fold). At no time was cortical GFAP staining altered by the injections. This result suggests that astrocytes may participate in neuroimmunological interactions. Supported by the American Heart Assn Grant-in-Aid 891079 and Established Investigator Award 890173 to DGM

499.5

EFFECT OF INTERLEUKIN 6 ON PROLACTIN SECRETION, cAMP AND INOSITOL PHOSPHATE PRODUCTION IN RAT PITUITARY CELLS. M.Grimaldi*,E.Landolfi*,T.Florio,O.Meucci* and G.Schettini. Dept. of Pharmacolgy, II School of Medicine, University of Naples, Via S. Pansini 5, 80131 Naples, ITALY.

Interleukin 6 (IL6) is a cytokine having many physiological activities. It has been reported that IL6 is released from rat Hypothalamus, suggesting a role for this cytokine in the regulation of pituitary hormone release. We analyzed the direct effects of IL6 on Prolactin (PRL) secretion on single lactotrophes, by means of reverse hemolytic plaque assay technique. IL6 (200-2000 U/ml) inhibited basal, VIP (-24%) and TRH (-20%) stimulated PRL secretion. Rat anterior pituitary cells pre-exposed to IL6 (500 U/ml) for 20 min showed a reduction of VIP and forskolin-stimulated adenylate cyclase activity (-15%), while the basal production of cAMP was not affected. Conversely when the cytokine was added directly to the cells membranes derived from pituitary no IL6 effect was detectable. Moreover pituitary cells pre-exposed to IL6 (5-500 U/ml) for 20 min showed a dose-dependent reduction of the TRH stimulated inositol posphate production (-25%). Our data suggest that IL6 modulates PRL secretion and affects the formation of both cAMP and inositol phosphate at pituitari level.

499.6

THE STIMULATORY EFFECT OF INTERLEUKIN-6 ON CORTICOTROPIN-RELEASING FACTOR AND THYROTROPIN RELEASING HORMONE SECRETION IN VITRO. K.Lyson, L.Milenkovic* and S.M.McCann. Department of Physiology, Neuropeptide Division, U.T. Southwestern Medical Center, Dallas, Tx 75235-9040.

Since we reported a stimulatory effect of intraventricularly injected interleukin-6 (IL-6) on ACTH release and an inhibitory influence on TSH secretion in vivo, we decided to determine the hypothalamic action of IL-6. After 30 min. preincubation rat medial basal hypothalamus (MBH) was incubated for 30 min.with IL-6 in Krebs-Ringer bicarbonate buffer, and corticotropin-releasing factor(CRF) and thyrotropin-releasing hormone (TRH) release into the incubation medium were measured by RIA. IL-6 (10^{-15}-10^{-9}) evoked a bell-shaped dose-dependent increased CRF secretion with a maximal effective dose of 10^{-13} M of IL-6 (Mean±SEM: 0.57±0.07, vs. control: 0.27±0.02, pg/mg/0.2ml, $p<0.005$), while TRH release was significantly stimulated only by a 10^{-11} M concentration of IL-6 (0.77±0.11 vs. control: 0.48±0.04, $p<0.025$). The results support a role of IL-6 at the hypothalamic level to stimulate CRF and to a lesser extent TRH release. (Supported by NIH grants DK 10073 and DK 40994.)

499.7

CENTRAL LIPOPOLYSACCHARIDE INFUSION INDUCES INTERLEUKIN-1 PRODUCTION IN THE ADULT RAT BRAIN IN VIVO. S.H. Lisanby, L.D. Rokoske, J.C. Ritchie, S.K. Sundar, and J.M. Weiss.

Interleukin-1 (IL1) is elevated in the cerebrospinal fluid during slow wave sleep, endotoxin-induced fever, and animal models of multiple sclerosis; however, it is not known whether IL1 enters the brain from the periphery (via the organum vasculosum laminae terminalis) or whether it is synthesized and released centrally. Previous studies have shown that neonatal astrocytes in vitro and injured brain biopsies release IL1; also, intraperitoneal (ip) lipopolysaccharide (LPS) has been reported to elevate brain and plasma IL1 and induce fever in the mouse. The objectives of this study were to determine whether endogenous IL1 production may be induced in the adult rat brain in vivo with LPS, and to investigate the role of central IL1 production in fever, steroidogenesis (SG), and immunosuppression (IS). LPS was infused into the lateral ventricles or ip, brain extracts were ultrafiltered and then fractionated to separate IL1 inhibitors. Ten and 100 ng LPS infused intracerebroventricularly (icv) significantly elevated IL1 bioactivity (17kd fraction) of glial lysates and brain interstitial fluid at 90 mins ($p<.05,.025$). After 24 hrs, brain IL1 was no longer elevated, coinciding with the reported time course for recovery from central LPS-induced fever. Plasma IL1 was not elevated after icv LPS. When given ip, 2.5 mg/kg LPS did not elevate brain IL1 at 5 hrs, but did elevate plasma IL1 and corticosterone ($p<.025$), results which suggest that SG reported after ip LPS may not result from elevated brain IL1. Interestingly, 1 hr of electric tail shock significantly elevated colonic temperature and plasma IL1, and pilot studies found no significant change in brain IL1 post shock; thus, findings after tail shock resemble effects observed after ip LPS injection. In conclusion, these results show icv LPS induces IL1 production in the adult rat brain in vivo with a time course paralleling that reported for central LPS-induced fever, suggesting LPS mediates fever via IL1. In that IS occurs 90 mins after 10 ng LPS icv, and icv LPS elevates brain IL1, the findings are consistent with the hypothesis that central IL1 production mediates IS following LPS in brain.

499.8

LOCALIZATION OF INTERLEUKIN-1 SYNTHESIS IN DIENCEPHALON REGION OF RATS CENTRALLY INFUSED WITH LIPOPOLYSACCHARIDE. S.K.Sundar, M.A. Cierpial, S.Long, J.C.Ritchie & J.M. Weiss
Dept. of Psychiatry, Duke Medical Center, Durham, NC 27710

Intracerebroventricular infusion of Interleukin-1 (IL1), a potent modulator of the hypothalamic pituitary adrenal axis, has been shown to produce rapid peripheral immunosuppression. Similar immunosuppression was also detected when de novo synthesis of IL1 was induced by central infusion of lipopolysaccharide (LPS) (Sundar et al 1989, PNAS 86:6398, 1989). It is of interest to determine which area(s) of brain produce IL1. Ninety minutes after central infusion of LPS, virus-antibody free Charles River rats were sacrificed and brains disected into four regions: cortex, cerebellum, diencephalon and brain stems of 3 rats were pooled together, and 3 whole brains were pooled. Astroglial cells were isolated, lysed by sonication, and the clarified supernatant was fractionated on Sephadex G-50 columns under aseptic conditions. Fractions were analyzed for IL1 by thymocyte proliferation test.

IL1 bioactivity corresponding to 17,000 kD was detected in extracts of whole brains and in diencephalon only; but not in the remaining brain regions. Thus LPS-induced IL1 was found in a region of brain that actively regulates HPA axis; consistant with this, plasma corticosterone was elevated in LPS-infused rats. These observations combined with the detection of IL1 mRNA will elucidate whether IL1 is synthesized locally or transported as a neurotransmitter.

499.9

INTERLEUKIN-1 (IL-1) INHIBITS ACETYLCHOLINE BIOSYNTHESIS IN CULTURED BASAL FOREBRAIN NEURONS. **L. Ni, R.P. Hart, and G. M. Jonakait. Dept. Biological Sciences, Rutgers University, Newark, NJ 07102**

Since brain levels of IL-1 are elevated in Alzheimer disease (Griffin et al.,*PNAS* 86, 7611, 1989), we sought to determine the possible influence of IL-1 on cholinergic neurons in both the basal forebrain (BF) and neostriatum (NS). Organ cultures of embryonic rat BF or NS (E16) were grown with and without human recombinant IL-1 for 8-10 days and choline acetyltransferase (ChAT) activity was measured as an index of cholinergic development. IL-1 (20 U/ml; Cistron) lowered ChAT activity in both brain regions by as much as 70%. In the BF, IL-1 did not affect levels of mRNA coding for neuron-specific enolase, suggesting that the effect of IL-1 was not a generalized toxic action on neurons in the cultures. Moreover, IL-1 had no effect on levels of message coding for glutamic acid decarboxylase, suggesting that GABAergic neurons were spared the effects of IL-1 and that the action on cholinergic neurons was somewhat specific. Cholinergic neurons, though compromised, survived a relatively brief (5-day) exposure to IL-1 since ChAT activity recovered to control levels following removal of IL-1. The addition of nerve growth factor following IL-1 removal stimulated ChAT activity still higher. These data suggest that IL-1 specifically and significantly compromises cholinergic biosynthesis in cultured BF and NS.

499.10

INTERLEUKIN-1 (IL-1) STIMULATES PREPROTACHYKININ GENE EXPRESSION IN CULTURED SYMPATHETIC GANGLIA. G. M. Jonakait and R. P. Hart, Dept. of Biological Sciences, Rutgers University, Newark, NJ 07102

Recent studies from our laboratory have shown that IL-1 dramatically increases substance P (SP) peptide in cultures of neonatal rat superior cervical (sympathetic) ganglia (SCG; Jonakait and Schotland, *J. Neurosci. Res.* 26:24-30, 1990). Northern blot analysis of RNA obtained from cultured ganglia show that mRNA coding for the prohormone precursor, preprotachykinin (PPT), is also increased 4-5-fold by treatment with IL-1, suggesting that increases in mRNA levels mediate the rise in peptide.

Since ganglia increase SP in response to deafferentation, we investigated the role played by depolarizing stimuli in the IL-1-induced increase in SP. Growth of SCG cultures in 40 mM KCl prevented IL-1-induced increases in either SP peptide or PPT mRNA, suggesting that deafferentation is required for immune responsiveness.

Glucocorticoid hormones suppress immune responsiveness in many systems. In this system, too, dexamethasone inhibited the ability of IL-1 to increase both SP peptide and PPT mRNA (K_i=5nM).

Indomethacin (1uM), included in the cultures to block IL-1-induced increases in prostaglandins, depressed the IL-1-induced increase in SP only slightly (21%), suggesting that prostaglandins are not primary mediators of this action of IL-1.

499.11

ENDOTOXIN OR INTERLEUKIN-1 ADMINISTRATION ELEVATES PLASMA CORTICOSTERONE, AND BRAIN MHPG AND TRYPTOPHAN. A.J. Dunn. Department of Pharmacology, Louisiana State University Medical Center, Shreveport, LA 71130-3932.

In previous experiments, administration of antigens or infection of mice elicited stress-like elevations of plasma corticosterone, and brain tryptophan, and increases in cerebral norepinephrine (NE) metabolism. Similar results were obtained following IP administration of various preparations of interleukin-1 (IL-1 human α or β, or murine α). We have now studied the endocrine and neurochemical responses to ICV IL-1, and IP or ICV endotoxin administration. ICV human IL-1α produced dose-dependent elevations of plasma corticosterone, and brain concentrations of tryptophan and the NE catabolite, MHPG. The effective dose range was not markedly different from that obtained with IP injections. This could imply that the effect of IP IL-1 is not due to penetration of the brain by IL-1. Two different preparations of E. coli lipopolysaccharide (LPS: Sigma L3129 and L3755) administered IP produced dose-dependent elevations of plasma corticosterone. Although responses were somewhat variable, doses > 0.10 μg were always effective, and L3755 was somewhat more potent than L3129. Accompanying the endocrine changes, were elevations of brain MHPG, the serotonin catabolite, 5-HIAA, and tryptophan. There were smaller elevations of the dopamine catabolite, DOPAC. ICV administration of LPS (0.1 to 5 μg) also elevated plasma corticosterone, and brain MHPG, but was less effective in elevating brain tryptophan. These results suggest that endotoxin, like foreign antigens and IL-1, can produce stress-like endocrine and neurochemical changes. This most likely occurs by LPS-stimulated macrophage secretion of IL-1.

Supported by grants from NIMH (MH 45270 and MH46261)

499.12

INTERLEUKIN-2 (IL-2) INHIBITS ACETYLCHOLINE RELEASE FROM RAT HIPPOCAMPAL SLICES BY ALTERING OPIOID PEPTIDE RELEASE D. M. Araujo, P. A. Lapchak, A. Beaudet, and R. Quirion. Douglas Hosp. Res. Ctr., Neuroanat. Lab., MNI, & McGill Univ., Montreal, Quebec, Canada.

Recently, we have shown that the lymphokine IL-2 decreases the K+-evoked release of acetylcholine (ACh) from rat hippocampal slices (Araujo et al., Brain Res. 498: 257, 1989). In the present study, we demonstrate that this effect is indirect and may be mediated by an interaction with opioid peptides, which are known to reduce ACh release in this tissue (Lapchak et al., Neurosci. 31: 313, 1989). First, inhibition of ACh release (by 31.5+4.2%) by IL-2 (10 nM) was prevented by tetrodotoxin (1 μM), suggesting that the effect of IL-2 is distal to the cholinergic terminals. Second, the effect of IL-2 appears to involve opiate receptors, since it was antagonized by naloxone (10 μM) and potentiated by B-endorphin (1 μM). Moreover, mu- opiate receptors are implicated, since neither kappa- nor delta- selective drugs alter ACh release in this tissue. However, a direct interaction of IL-2 with opiate receptors seems unlikely since naloxone (10 μM) did not alter the binding of IL-2 to its receptor sites in the hippocampus. Furthermore, IL-2 increased (by 24-44%) the release of endogenous opioid peptides (met-enkephalin and leu-enkephalin) from slices. Thus, it appears that in the hippocampus IL-2 inhibits ACh release indirectly, by enhancing the release of endogenous opioid peptides. (MRC & FRSQ, Canada)

499.13

IMMUNOAUTORADIOGRAPHIC LOCALIZATION OF INTERLEUKIN-2 RECEPTORS (Tac ANTIGEN) IN RAT AND HUMAN BRAIN. A. Beaudet, D.M. Araujo, R. Quirion and P.A. Lapchak. Neuroanatomy Lab., Montreal Neurological Institute and McGill University, Montreal, Quebec, H3A 2B4, Canada.

Recent evidence suggests that the lymphokine interleukin-2 (IL-2) may be involved in the regulation of CNS function. In the present study, we determined the localization of IL-2 receptor immunoreactivity (IR) in sections of rat and human brain using the monoclonal antibody anti-Tac. In rat brain, Tac IR was observed in a limited number of regions including the hippocampal formation, median eminence-arcuate nucleus complex, cerebral cortex (lamina IV), lateral septum, neostriatum and cerebellum. This distribution was comparable to that of IL-2-like IR, suggesting that IL-2 may be synthesized and/or stored in the vicinity of interaction with its receptor. In sections from human hippocampal formation, Tac IR was detected throughout the hippocampus and dentate gyrus. Moreover, brains from patients with Alzheimer's disease exhibited qualitatively higher immunostaining of the dentate gyrus than those of controls. In summary, our results demonstrate that IL-2 receptors are present in both normal and diseased mammalian brain. These results suggest that IL-2 may be involved in immune responses during neurodegenerative processes, in addition to playing a role in the regulation of neuronal function in the CNS. (Supported by MRC, Canada and FRSQ, Quebec).

499.14

IMMUNOHISTOCHEMICAL DETECTION OF INTERLEUKIN-2 IN NORMAL MOUSE BRAIN. F. Villemain, J.M. Girard*, T. Owens* and A. Beaudet. Neuroanatomy Lab., Montreal Neurological Institute, Montreal, Quebec, H3A 2B4, Canada.

Previous studies have suggested that interleukin-2 (IL-2), a lymphokine produced by activated T cells and playing a central role in immune cooperation mechanisms, may be present in mammalian brain. In the present study, endogenous IL-2 was detected by immunohistochemistry in the brain of normal CD-1 mice. Labeling was performed on frozen sections using a rat IgG2a anti-murine IL-2 (S4B6) as primary antibody and either peroxidase anti-peroxidase or immunoautoradiography as detection procedures. In brains fixed by intracardiac perfusion of a 4% paraformaldehyde solution, dense Il-2 immunoreactivity was detected within the arcuate nucleus-median eminence complex of the hypothalamus as well as over capillary walls throughout the brain. Animals perfused with a mixture of 4% paraformaldehyde and 0.2% or 0.5% glutaraldehyde exhibited even stronger labeling of the arcuate nucleus-median eminence complex but were virtualy devoid of vascular staining. Lymph nodes from the same animals exhibited positive IL-2 immunostaining within a selective population of T cells at the periphery of secondary follicles, irrespective of the fixation conditions. These studies provide the first demonstration of the presence of IL-2 immunoreactivity in adult mouse brain and indicate that most of the lymphokine is concentrated within the arcuate nucleus-median eminence complex of the hypothalamus. Supported by MRC.

499.15

IDENTIFICATION OF INTERLEUKIN-1 RECEPTOR mRNA IN MURINE HIPPOCAMPUS. E. Wada*, E.T. Cunningham, Jr., Wm. M. Mitchell, D.B. Carter*, D.E. Tracey*, J.F. Battey, and E.B. De Souza. Neurobiology Laboratory, NIDA Addiction Research Center, Baltimore, MD 21224 (E.T.C. Jr., W.M.M., E.B.D.S.); Laboratory of Neurochemistry, NINDS, Bethesda, MD 20892 (E.W., J.F.B.) and The Upjohn Company, Kalamazoo, MI 49007 (D.B.C, D.E.T.).

The cytokine interleukin-1 (IL-1) has been reported to have a number of effects in brain including induction of fever, alteration of slow-wave sleep and neuroendocrine effects such as activation of the hypothalamic-pituitary-adrenal axis and inhibition of the hypothalamic-pituitary-gonadal axis. In this study, we sought to identify the brain areas that might mediate some of these effects of IL-1 by using in situ hybridization of ^{35}S-labeled anti-sense cRNA probes derived from a full-length murine T-cell IL-1 receptor cDNA to investigate the distribution of IL-1 receptor (IL-1-R) mRNA in the mouse brain. In general, the level of IL-1-R mRNA signal was very low, with the molecular layer of the dentate gyrus of the hippocampus providing the only prominent signal in brain. Notably, the signal in the hippocampus was much higher than that found in the hypothalamus, a brain area implicated in the effects of IL-1 on neuroendocrine function. Control sections hybridized with sense cRNA probes displayed no signal above background. The pattern of distribution of IL-1-R mRNA in brain was similar to the autoradiographic distribution of ^{125}I-IL-1α-labeled IL-1 receptors. These results suggest that IL-1 may alter neuroendocrine activity to a large extent through actions in the hippocampus in a manner similar to glucocorticoid regulation of hypothalamic hormone secretion.

499.16

INTERLEUKIN-1 RECEPTORS IN MOUSE BRAIN: CHARACTERIZATION AND AUTORADIOGRAPHIC LOCALIZATION. T. Takao, D.E. Tracey*, W.M. Mitchell and E. B. De Souza. NIDA, Baltimore, MD 21224; The Upjohn Co., Kalamazoo, MI 49007.

The cytokine interleukin-1 (IL-1) has a variety of effects in brain including induction of fever, alteration of slow-wave sleep and alteration of neuroendocrine activity. The potential sites of action of IL-1 in brain were examined using ^{125}I-recombinant human interleukin-1α (^{125}I-IL-1α) to characterize IL-1 receptors in homogenates of mouse (C57BL/6) hippocampus and to localize IL-1 binding sites using autoradiography. The binding of ^{125}I-IL-1α was linear over a broad range of membrane protein concentrations, saturable, reversible, and of high affinity (K_D: 114±35 pM; B_{max}: 2.5±0.4 fmol/mg protein). In competition studies, recombinant human IL-1α, recombinant human IL-1β and a weak IL-1β analog IL-1β^+ inhibited ^{125}I-IL-1α binding to mouse hippocampus in parallel with their relative bioactivities in the T cell comitogenesis assay with K_i values of 55±18, 76±20 and 2940±742 pM, respectively; rat/ human corticotropin-releasing factor and human tumor necrosis factor showed no effect on ^{125}I-IL-1α binding. Autoradiographic localization studies revealed very low densities of ^{125}I-IL-1α binding sites throughout the brain, with highest densities present in the molecular and granular layers of the dentate gyrus of the hippocampus and in the choroid plexus. The identification of IL-1 receptors in brain with characteristics similar to IL-1 receptors in immune and neuroendocrine tissues provides further support for a physiological role for IL-1 to regulate central nervous system activity.

500.1

LAMINAR DISTRIBUTION OF ZINC IN THE RAT SmI BARREL CORTEX. N. D. Akhtar and P. W. Land. Dept. of Neurobiology, Anatomy and Cell Science, Univ. of Pittsburgh, Sch. of Med., Pittsburgh, PA 15261.

We used the selenium method of Danscher (Histochemistry (1982) 76:281-293) to localize zinc storage granules in the rat somatosensory (SmI) barrel cortex. The distribution of selenide-precipitated zinc was compared with staining for cytochrome oxidase activity. There is a heterogeneous distribution of zinc staining in rat SmI cortex. The darkest staining is in lamina V which stains non-uniformly for zinc. Lamina V contains dark patches of zinc staining that tend to fall below the septae between barrels in lamina IV. Lamina IV itself is organized into lightly stained patches, each of which is centered upon a barrel. Intervening septal regions are more darkly stained. A band of light staining is present at the lamina V/VI border. Lower lamina VI and laminae II/III have moderate levels of staining, whereas lamina I is only lightly stained. Several studies suggest that zinc is released at excitatory cortical synapses. In addition, it is thought that zinc modulates neurotransmission at glutamate receptors. Thus the pattern of zinc staining in the barrel cortex may reflect functional differences in excitatory input to different regions within SmI. (Supported by NIMH grant MH09773 and NIH grant NS 23047).

500.2

METABOLIC CORRELATES OF SENSORY DISUSE AND RECOVERY DURING AGING. J. Metzler and P.J. Hand. Dept. of Animal Biol., Univ. of Penn. Sch. Vet. Med., Phila., PA 19104.

Effects of chronically altered sensory input on the aging CNS and the time course of any changes examined. The rat vibrissal SI cortical barrel system model was used. Effects of disuse and subsequent recovery of single vibrissa C3, on C3 and adjacent vibrissae B3, C2, C4, and D3 functional representations in lam. IV of SI was examined using the (^{14}C)-deoxyglucose (2DG) method. Twenty four young adult (YA;90-da old) and 6 aged (AG; 1.5-2 yr old) rats were studied. Two groups of 12 YA had C3 clipped (disuse) unilaterally for 30 or 90 da. Control side C3 was clipped or not during this time. In 6 animals in each YA group, C3 regrew (recovery) for 30 da. Surrounding B3, C2, C4 and D3 vibrissae were spared and stimulated or not following 2DG injection. Of 6 AG rats examined, C3 was clipped unilaterally--4 for 30 and 2 for 90 da; 3 rats had B3, C2, C4, and D3 stimulated during the 2DG procedure. Analyses indicated that in YA and AG animals 1) B3, C2, C4, D3 vibrissae activation produces increased labeling in the "disused" C3 representation as compared to control side; 2) 30 and 90 da disuse effects are similar; 3) labeling was similar in control and "recovered" C3 representations; and 4) compensatory changes are greatest in YA. In conclusion, while the YA CNS is capable of greater functional responses to sensory disuse and recovery, the AG CNS also exhibits plasticity. (Supported by U. PA. Res. Found. Grant & USPHS Grant NS-22283.)

500.3

VIBRISSA DENERVATION EFFECTS ON CORTICAL REPRESENTATION OF SUPRAORBITAL (SOG) AND INFRAORBITAL (IOG) GUARD HAIRS IN RAT. P.J. Hand, S-C.Sheu, and F-J Lai *. Dept. of Animal Biol., Sch. of Vet. Med., Univ. of Penna., and Dept. of Anat., Natl. Yang-Ming Med.Col., R.O.C.

Does peripheral denervation alter the principal cortical target (PCT) of a spared sensory receptor organ, which expanded its representation into denervated territory, but is at a distance from the surgical field? Six 2-day old rats received unilateral vibrissa denervation. 90 days post-denervation, 14C-deoxyglucose (2DG) was injected and either SOG (n=3) or IOG (n=3) stimulated bilaterally. SOG and IOG activation on the non-denervated side served as a control. 2DG labeling [cerebral glucose utilization rates (CGU) and areal extent] was analyzed within lam.IV of SI posteromedial barrel subfield (PMBSF) and adjacent SOG-IOG PCTs. Results (in comparison to control side) were as follows: (1) SOG and IOG activation produced a 29.5% increase in CGU in caudal-half of PMBSF overlying rows B-E and associated "straddlers" [mean areal increase of labeling beyond their PCT was 45% (SOG) and 119% (IOG); (2) SOG and IOG labeling in register with the PCTs exhibited no LCGU differences ($p>.05$); and (3) mean areal extent of labeling associated with PCTs were altered- 40% decrease (SOG) and 44% increase (IOG). Thus a peripheral receptor organ's PCT representation can be effected (e.g., areal extent altered) by a "distant" peripheral denervation and may significantly influence the recovery process. (Supported by USPHS grant NS-22283 and R.O.C. Natl. Sci. Council grant)

500.4

DEVELOPMENT OF LAYER IV IN RAT SOMATOSENSORY CORTEX L. Zhang and N. G. F. Cooper. Dept. of Anatomy and Neurobiology, Univ. of Tenn., Memphis, TN 38163

Layer IV of the somatosensory cortex of rodents contains a characteristic pattern of neuronal aggregates called barrel subfields. The objective of this study is to relate patterns of thalamocortical afferents to barrel subfield patterns during development. We have investigated the development of the rat somatosensory barrel field cortex with cresyl violet and carbocyanine dye DiI as follows:(1) Cresyl violet staining of tangential sections of SmI cortex on postnatal day 2(P2) through P11. (2) Thalamocortical mapping with DiI in postmortem fixed tissue from P1(day of birth) to P9. We found that granule cells in layer IV of SmI cortex appear as a continuous sheet prior to P3. On P4 separations in this sheet allowed visualization of cortical subfields representing face, forelimb, hindlimb and trunk. Nissl stained barrels were detected on P4 in the face area. Barrels representing forelimb and hindlimb were visible between P5-P6. DiI labeled thalamocortical axons reached the marginal zone on P1 and appeared to be sorted in layer IV of the cortical-face region by P3. Thus, the pattern of thalamocortical afferents from VPM appears before the corresponding cortical barrels representing the face. Further studies are required to determine if this sequence is repeated for forelimbs and hindlimbs. The segregation of thalamocortical axons appears to be concurrent with the appearance of glial patterns, but not with neuronal patterns. Supported by NIH:NEI EY02708.

500.5

THALAMIC AFFERENT SEGREGATION PRECEDES BARREL FORMATION IN THE RAT SI CORTEX. R.S.ERZURUMLU AND S.JHAVERI Dept. Brain & Cognitive Sci., M.I.T., Cambridge, MA 02139.

The formation of periphery-related patterns by somatosensory thalamocortical afferents was examined in paraformaldehyde-fixed embryonic and neonatal rat pups. Axons from the ventro-basal (VB) nucleus to somatosensory SI cortex were labeled with the fluorescent tracer DiI.

VB afferents are present in the cortical plate (CP) by E19. On PND 0 (=E22), layers V and VI have differentiated from the CP, and VB axons form a dense plexus within these layers; a few thalamic axons traverse the CP and extend into the marginal zone. By PND1, VB axons define their projection zone in the lower part of the CP. However, no patchy distribution of thalamic afferents is evident at this time. On PND 2, a vibrissa row-related pattern with occasional patches emerges within the presumptive layer IV. This pattern sharpens over the course of the next 24 hours and by PND 3, individual vibrissa-related patches can be clearly distinguished.

Thus, a vibrissa-related punctate organization in SI cortex is established by VB axons prior to differentiation of layer IV cells into barrels. However, maturation of this pattern may not be independent of other cortical elements (see Blue et al., *Soc. Neurosci. Abstr.*, 1990). Supported by NIH grant NS 27678.

500.6

NEONATAL P-CHLOROAMPHETAMINE (PCA) TREATMENT DELAYS PATCH FORMATION BY THALAMOCORTICAL AXONS IN RAT BARREL CORTEX. M.E. Blue, R.S. Erzurumlu, S. Jhaveri and M.V. Johnston. The Kennedy Institute and Johns Hopkins Medical Institutions, Baltimore, MD 21205 and Massachusetts Institute of Technology, Cambridge MA, 02139.

During early postnatal development, dense patches of serotonergic (5-HT) axons from the raphé nuclei form a vibrissa-related pattern in barrel field cortex. Afferents from the ventrobasal (VB) nucleus display a similar pattern which develops just prior (12-24 hrs.) to that of 5-HT axons. The present study examines a possible interaction between the two cortical projections. Rats were administered the 5-HT neurotoxin, p-chloroamphetamine, (PCA; 10 mg/kg, s.c.) on postnatal days P0, P1, P2 and P3; control littermates received saline. Pups were sacrificed on P2.5 and 3.5. Thalamocortical axons were labeled with the fluorescent tracer, dil and their distribution examined in flattened cortex preparations.

Normally, the vibrissa-related pattern of VB afferents emerges on P2. In PCA-treated rats, the development of this pattern lags one day behind controls. The density of 5-HT axons in the VB is low in both groups, indicating that the effect is mediated at a cortical level. These results along with previous findings of a delay in the formation of barrel cytoarchitecture after PCA-treatment suggest a trophic influence by serotonin on maturing VB afferents and on the layer IV cells that comprise the barrels.

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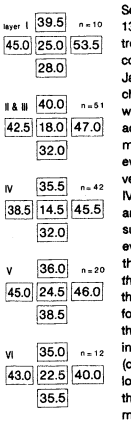
500.7

Physiological aspects of the barrel cortex of the mouse: a single unit laminar latency analysis.

E. Welker*, M. Armstrong-James+, H. Van der Loos*.

* Institute of Anatomy, University of Lausanne, Rue du Bugnon 9, 1005 Lausanne, Switzerland.
+ Dept. Physiology, London Hospital Medical College, London E1 2AD, U.K..



Serving as a baseline for plasticity studies, we have been recording from 135 single units in the barrel cortex of normal mice. Carbon fiber electrodes were lowered perpendicular to the pial surface into barrel columns B2, B3, C1, C2, C3 and D2 (for technical details see Armstrong-James and Fox, 1987, J Comp Neurol 263:265-281). The response characteristics of units in layer IV were used to determine the principal whisker (PW) for a given penetration. Units were pooled, and classified according to cortical layer. The numbers in the figurines indicate the median latencies for responses of the PW (central blocks) and of those evoked by stimulation of surrounding whiskers (rows displayed vertically; arcs, horizontally). The shortest latency was observed in layer IV upon stimulation of PWs. Responses to surround whiskers in the row arrive earlier than to stimulation of surround whiskers in the arc. In supragranular layers the rostral surround whisker in the row (lower block) evoked shorter latency responses than the caudal whisker in the row; for these layers a similar anisotropy is observed within an arc: signals from the more lateral surround whisker (left block) reach the cortex faster than those from the whisker medial to PW. In the infragranular layers these forms of anisotropy do not seem to exist. Comparing these data with those obtained in rat (article quoted above), we conclude that information from surrounding whiskers elicits in the mouse much later (ca. 2x) cortical responses than in the rat. The mechanism behind this longer period of "reflection" can be of subcortical or intracortical nature; this difference can be considered as a new piece of evidence that a mouse is not a rat. Support: Swiss NSF 3100.009468.

500.8

Intra-columnar and interlaminar relay of vibrissal inormation in barrelfield cortex.

By *M. Armstrong-James and **K. Fox. *London Hospital Medical College, Turner St., London E1 2AD, U.K. and **Center for Neural Science, Brown University, R.I. 02912.

Our previous studies have shown that surround receptive fields of barrel cells in rat S1 cortex appear to be constructed intracortically. We now report that the centre receptive vibrissa (CRV) data is relayed vertically in a column of neurons prior to relay to adjacent columns. Extracellular recordings were made in identified layers of rat barrel cortex and latency differences of neurons to stimulation of centre and surround receptive vibrissae (CRV and SRV) measured. Latency differences for pairs of cells in the same penetration to stimulation of the CRV were also collected. To the CRV, Layers IV and Vb neurons discharged earliest, and layers II and III on average 2-3 ms later. The data suggests that serial relay from layers IV to III to II is the most common event. No significant differences were found for latency or magnitude of response of layer Va cells to stimulation of centre and immediate SRVs. *Temporal summation of responses to several* simultaneously stimulated vibrissae is therefore ideal in layer Va. Layer II, III, IV and Va cells showed no statistical difference in latency of discharge to SRVs, suggesting parallel column-column relay for construction of surround receptive fields. The mean transmission velocities were calculated at about 0.08 m/s for column-column information transfer and 0.18 m/s for intracolumn (layers IV-II-II) transfer (assuming major relay from layer IV).

500.9

ECTOPIC LOCATION OF HEAVILY 2DG-LABELED NEURONS IN BARREL CORTEX OF THE BEHAVING HAMSTER

J.S. McCasland, R.W. Rhoades, and T.A. Woolsey. Division of Experimental Neurology and Neurosurgery and McDonnell Center for Studies of Higher Brain Function, Washington University. School of Medicine, St. Louis, MO 63110, and Department of Anatomy, Medical College of Ohio, Toledo, OH 43699

We used a high-resolution 2-deoxyglucose (2DG) technique in material double-labeled for cytochrome oxidase (CO) to study stimulus-dependent patterns of label in barrel cortex of the behaving hamster. We compared the distribution of 2DG label in cortices of normal behaving animals with those in which all large whiskers except left C3 and right A1 and E4 were trimmed one day before the experiment. In normals, all barrel columns were heavily 2DG labeled. The whisker-trimmed animals showed increased labeling mainly in the barrel columns appropriate to the spared whiskers in both cortices as well as in the brainstem and thalamic representations of those whiskers. A small subset of cortical neurons, scattered through lower layer IV and upper layer V and with characteristics of nonpyramidal GABAergic neurons, showed strikingly heavy labeling. Interestingly, in whisker-trimmed subjects many heavily labeled neurons were found "ectopic" to the principal columns of the stimulated whiskers. The locations and numbers of these cells were highly variable from case to case, but in each case examined to date showed a rough correspondence in spatial distribution to the principal whisker columns. The zone of heavy 2DG labeling invariably corresponded precisely to heavily CO-stained somata. Studies are under way to determine whether these cells are indeed GABAergic. The ectopic locations of many heavily-labeled neurons, in contrast to the homotopic labeling seen in brainstem and thalamus, suggests that these neurons function as a neural network involved in regulation of intracortical activity. Supported by NIH grant NS17663.

500.10

PRENATAL CENTRAL VIBRISSAL PATHWAYS LABELED WITH DiI AND DiA

S. L. Senft. *Department of Neurology and Neurosurgery, Washington University School of Medicine, Saint Louis, Mo. 63110.*

Earlier work focused on postnatal elaboration of mouse thalamocortical axons (Senft, 1987). This pathway (and also the cortico- and trigemino- thalamic projections) is now being studied in prenatal mice. Fluorescent lipophilic dyes, the red-orange "DiI" and the yellow-green "DiA" (Molecular Probes), are applied to fixed cortex, thalamus and brainstem, and the tissue is stored at 37-60 C° for 4-14 days.

The Ventro-Basal (VB) nucleus is retrogradely labeled from cortex as early as E17 (E0 indicates conception; ~E19 the day of birth), when fibers from the brainstem trigeminal complex are within the diencephalon.

Several technical items are notable: (1) Perfusion is with unbuffered 8% paraformaldehyde (pH 10), producing hardened tissue that sections uniformly and contains very crisp dye labeling. (2) For cytoarchitecture, 0.05% bisBenzimide (Molecular Probes) is added to the perfusion fixative, staining the brain uniformly *en bloc* and obviating any extra manipulations that might damage sections. (3) DiI and DiA are solubilized with dichloromethane (Aldrich) and pressure-ejected from micropipettes. (4) Cells and fibers remain distinct for weeks to months, at room temperature, mounted in a 1:1 solution of 100% glycerol and 8% paraformaldehyde (pH 8-10). (At lower pH labeled membranes become fuzzy shortly after sectioning and clearing.) (5) Vibratome sections cut in this solution are already cleared and can be mounted directly. Supported by NIH grants NS 17763 and NS 07057.

500.11

EFFECTS OF EARLY WHISKER REMOVAL ON THE DEVELOPMENT OF THE PROJECTIONS FROM ZONA INCERTA TO THE NEOCORTEX.

M.A.L. Nicolelis, C-S.Lin and J.K. Chapin. Hahnemann Univ., Phila., PA 19102.

Recently we described a major GABAergic projection from zona incerta (ZI) to the entire neocortex of rats that is remarkably expanded in young animals. In the present study we examined the development of this projection and its alteration by early postnatal (PN) whisker removal. Long-Evans rats (n = 50) ranging in age from PN day 1 to adulthood received small injections (0.1 to 0.5 µl) of either *rhodamine coated microspheres (RCM) or Fluoro-gold (FG) in the primary* somatosensory (SI) and/or primary visual cortex. The projection from ZI to SI was first detected in normal animals injected at PN day 1 and perfused at PN day 3. It reached its maximal expansion at PN week 1, remained stable until PN week 2 and started to decline after PN week 3 until it reached its adult pattern. Another group of animals (n = 17) was subjected to monolateral whisker removal at PN day 1. After reaching adulthood these animals received small injections of fluorescent tracers in the SI cortex (barrel fields). These injections were found to label as high a proportion of neurons in the ZI of these whisker deprived adults as in normal young rats (1 to 2 weeks old). In addition, projections from the magnocellular subdivision of the medial geniculate body to the SI (previously identified only in young rats, but not in normal adults) were also observed in these deprived animals. Neurophysiological mapping of somatosensory responses in the ventral posteromedial (VPM) thalamus were also carried out in neonatally deprived adult animals anesthetized with pentobarbital. Unlike normal adults, neurons in the *VPM of these deprived rats were found to respond preferably to ipsilateral* manipulation of the whiskers and secondarily to other receptive fields located in the contralateral face. Overall, these results indicate that early postnatal whisker removal can produce a stabilization of otherwise transient thalamocortical and incertocortical projections. *Supported by grants NS26722, AAO6965, KO2-AA00089, AFOSR 90-0266 and FAPESP 88/4044-9.*

500.12

TOPOGRAPHIC MAPPING OF EVOKED SOMATOSENSORY POTENTIALS IN RAT: SPATIAL AND TEMPORAL ANALYSIS.

SHI DI and DANIEL S. BARTH *Departments of Neurology and Psychology, Univ.of California, Los Angeles, (U.S.A)*

Surface mapping of evoked potentials in vibrissa/barrel cortex has been studied in rat (Woolsey, 1948; Axelrad,1976). The present study reexamined the topographic vibrissal representation in rat SmI using an 8x8 multichannel *microelectrode array to simultaneously record* epicortical field potentials. The potential complex evoked by vibrissal displacement began with a positive-negative fast wave followed by a positive-negative slow wave, which were defined sequentially as P1, N1, P2, and N2 components. The P1 and N1 components, with latencies of ~8 and ~15 ms respectively, were spatially restricted within a 1 mm² cortical area and demonstrated a vibrissal dependent topology similar to previous cortical recordings, corresponding to the spatial arrangement of layer IV barrels. In contrast, the P2 (~30 ms) and N2 (~90 ms) were widely distributed over 2-3 mm² recording areas, and had a vibrissa dependent topology that only approximated that of the P1 and N1. These data indicate that the fast activation (P1 and N1) spreads from the corresponding barrel focus over a short period and obscures the clear somatotopic map seen at earlier latencies. *Our results suggest that the evoked response complex consists of* individual temporal components with distinct spatial distributions. Spatial and temporal analysis of the surface response complex, particularly if combined with selective laminar potential measurements, may provide a useful approach for studying vibrissal information processing at the neural network level.

500.13

NUCLEUS BASALIS NEURONS PROJECTING TO PRIMARY SOMATOSENSORY CORTEX BARRELS. M.A. Howard,D.J. Simons,E.W. Rubel. Depts. Neurosurg. and Otolaryngol., U.Wash., Seattle WA 98195 and Dept. of Physiol., U. Pittsburgh, Pittsburgh, PA 15261

Retrograde tracing experiments were conducted to localize Nucleus Basalis Magnocellularis (NBM) neurons projecting to somatosensory cortex barrels. Barrels were mapped under physiologic control using a tungsten microelectrode. Rhodamine dextran was injected into one barrel per rat. Rats were sacrificed 3 to 6 days later. Cytochrome oxidase staining confirmed barrel field injection sites encompassing no more than 2 barrels. Thalamic ventrobasal complex (VB) cells labeled intensely in all rats. Labeling of NBM neurons was less intense and improved with longer post-injection survival times. Labeled neurons consistently occupied a region of anterior ventral NBM extending caudally approximately 1.5 mm from the anterior commissure. Labeled cells within each coronal section were located within the ventral portion of NBM, 1.5 to 3.0 mm from the midline. The maximum number of labeled cells per coronal section was 7 and 28 for NBM and thalamic neurons respectively. Total number of labeled NBM neurons was variable (range 1-44) despite consistent labeling of BV neurons.

Barrelfield projecting neurons of NBM are located within a discrete region of the nucleus and may be selectively manipulated with a stereotaxic probe. (Supported by NIH Grant NS08438-01).

SENSORY SYSTEMS—RETINA: RETINAL GANGLION CELLS

501.1

THE DISTRIBUTION OF RETINAL GANGLION CELLS IN NORMAL (CD-1) AND TRANSGENIC MICROPHTHALMIC MICE. S.D. Schlussman and S.C. Sharma. Depts. Cell Biol. and Anatomy and Ophthalmology, New York Medical College, Valhalla, NY 10595.

Genetic ablation of gamma-2 crystalline expressing cells leads to reduction in lens size and these transgenic animals have a microphthalmic phenotype (Breitman et al, Science 238:1987). In order to evaluate the effects of microphthalmia on the number and distribution pattern of retinal ganglion cell layer (GCL) neurons, cell counts and isodensity maps were made from flat-mounted retinae of control (CD-1) and transgenic microphthalmic mice. Isodensity maps revealed a centroperipheral gradient with the highest cell density just temporal to the optic disk and the lowest in the far periphery. The cell density was lower in microphthalmic mice than controls at all retinal eccentricities. Estimates of the total number of neurons in the GCL revealed that there were 70% fewer neurons in the microphthalmic GCL compared to control (33,000 vs. 113,000). We demonstrated earlier that transgenic microphthalmic mice contained 70% fewer optic axons than controls (8,500 vs 52,500). Comparison of these studies suggests that a high proportion of the neurons in the GCL of transgenic animals may be displaced amacrine cells.

501.2

LUCIFER YELLOW, FLUORESCENT RETROGRADE TRACERS AND FRACTAL ANALYSIS CHARACTERISE FERRET RETINAL GANGLION CELLS. R.J.T.Wingate*, T.FitzGibbon*, E.I.Webb* and I.D.Thompson*. (SPON: Brain Research Association). University Laboratory of Physiology, Parks Road, Oxford OX1 3PT, United Kingdom.

We have used an in vitro method to study the morphology of adult ferret retinal ganglion cells. Fluorescent retrograde tracers were injected into the lateral geniculate nucleus and/or the superior colliculus, some days prior to the light perfusion fixation and isolation of the retina. Labelled cell bodies were identified and intracellularly injected with lucifer yellow under a fixed stage compound microscope equipped with epifluorescence. By selective use of different coloured tracers, the branching pattern of the retinofugal axons could also be assessed. Lucifer yellow fills were drawn from serial photographs. Drawings were digitised on a 256 by 256 pixel array (Seescan Imaging Limited, Cambridge, U.K.) and the dendritic branching pattern analysed. Ganglion cell morphology resembled the alpha, beta and gamma classes, confirming the tracer studies of Vitek et al, 1985 (J.Comp.Neurol.,**241**,1-11). Quantitative comparisons of dendritic morphology were undertaken using automated Sholl ring counts, supplemented by a routine enabling the fractal dimension (df) of the arbors to be estimated (Caserta et al, 1990, Phys.Rev.Lett.,**64**,95-98). Gamma-like cells are found to have a substantially lower fractal dimension to both alpha and beta-like types. Alpha, beta and gamma cells have characteristicly differing central projections.

501.3

MORPHOLOGICAL CHARACTERISTICS OF NEOTENOUS TIGER SALAMANDER GANGLION CELLS. C.B.Toris, F.F. Risinger* and R.F. Miller. Dept. Physiol. Univ. of MN, Mpls, MN 55455.

Tiger salamander retinal ganglion cells were examined morphologically by injection of horseradish peroxidase (HRP) into the optic nerve sheath, followed by computer aided neuron reconstruction of the HRP filled cells. Based on cell size, placement and dendritic complexity, four broad types were identified to date (small-simple, SS; small-complex, SC; large-simple, LS; and large-complex, LC). Cells having an area of influence of less than 30,000 μm^2 were considered small. Cells with less than 35 terminal dendritic branches and/or short dendritic segment lengths (less than 15 μm) were considered simple. LC cells had a circular dendritic branching pattern extending throughout the inner plexiform layer (IPL). The dendrites of LS cells branched in a polar pattern and arborized in either sublamina a and/or b of the IPL. SC cells had dendrites arborizing in the IPL in sublamina a and/or b. SS cells differed from SC cells by having smaller total dendritic fiber length and a smaller average number of terminal dendritic branches (18 vs 45, respectively). The density of ganglion cells was estimated at approximately 300 cells/mm^2. Soma density and size were independent of eccentricity. Displaced ganglion cells were rare (2 of 58). These findings show that the ganglion cells of the tiger salamander differ dramatically from those of the mudpuppy retina. Detailed structure/function correlations will be necessary to further characterize ganglion cells in these animals. (R01EY07376)

501.4

PEPTIDERGIC DISPLACED GANGLION CELLS IN THE PIGEON RETINA. D.E. Hamassaki* and L.R.G. Britto. Dept. Physiol. Biophys., Inst. Biomed. Sci., São Paulo State Univ., 05508 São Paulo, SP, Brazil.

Displaced ganglion cells (DGCs) represent the sole source of retinal axons entering the nucleus of the basal optic root (nBOR) of the avian accessory optic system. In this study we investigated chemically-specific DGCs projecting to nBOR. Retrograde labeling of DGCs was obtained with rhodamine beads injected into nBOR of pigeons anesthetized with ketamine and xylazine. The indirect fluorescence and avidin-biotin methods were used to detect immunoreactivity to antibodies against substance P (SP, Sera Lab) and cholecystokinin (CCK, Immunonuclear). Following rhodamine beads injections into nBOR, approximately 4,700 DGCs were retrogradely labeled in the contralateral retina. About 25% of these DGCs were also labeled with the antibody against SP. A different population of about 15% of the DGCs projecting to nBOR showed CCK immunoreactivity. Both types of DGCs were mainly found in peripheral retinal regions and their sizes ranged from 12-24 µm (SP) and 18-29 µm (CCK). Together with previous data on catecholaminergic DGCs, the present results indicate that retinal inputs to nBOR are chemically heterogeneous.

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501.5

GABAERGIC GANGLION CELLS IN CAT RETINA L. Shelton*, S.B. Tieman and K.R. Fry. Neurobiology Research Ctr, SUNY, Albany NY 12222 and Ctr for Biotechnology, Baylor College of Medicine, The Woodlands TX 77381.

Until recently, little was known about the neurotransmitters used by the ganglion cells of the vertebrate retina. We have recently shown that whereas most retinal ganglion cells (RGCs) contain N-acetylaspartyl-glutamate (NAAG), 5-10% do not. Gamma-aminobutyric acid (GABA) has previously been localized in a small percentage of RGCs in the rat and the rabbit. To determine whether any RGCs in the cat contain GABA, we have attempted to double-label these cells with antisera to GABA and with AB5, a monoclonal antibody specific for RGCs. Six cats were deeply anesthetized and perfused with a mixture of 4% paraformaldehyde and 4% carbodiimide. Retinal sections or whole-mounts were incubated in a mixture of the primary antibodies (guinea pig anti-GABA (Chemicon) at 1:1800; AB5 at 1:7500), followed by rhodamine-labeled goat anti-guinea pig and fluorescein-labeled goat anti-mouse (Jackson; each at 1:100). As previously reported, AB5 labeled many cell bodies in the ganglion cell layer (presumably RGCs) and their processes in the inner plexiform and optic nerve fiber layers, while the GABA antibody labeled numerous small cells in the inner nuclear and ganglion cell layers (presumably amacrine cells) as well as a few larger cells in the ganglion cell layer. The great majority of labeled cells contained only one label. However, in every cat tested so far, a few cells (up to 5% of the AB5-positive cells) contained both labels, suggesting that in cat, as in rat and rabbit, some RGCs are GABAergic. (Supported by NSF grant BNS-8811039 to SBT and PHS grant EY06469 to KRF.)

501.6

NMDA-evoked responses in retinal ganglion cells of the larval tiger salamander. **J. Gottesman & R.F. Miller Dept. Physiology & Graduate Program in Neuroscience, Univ. of Minnesota, Mpls. MN 55455.**

We measured *N*-methyl-D-aspartate (NMDA) evoked currents via whole cell recordings made in a retinal slice preparation using the larval tiger salamander. We recorded ganglion cell responses to bath applications of agonists and antagonists in both current- and voltage-clamp. All cells displayed an inward current to NMDA when in normal (1 mM) external Mg^{2+} and held at -60 to -70 mV. This current reversed near 0 mV and had a negative slope conductance region beginning at potentials around -30 to -40 mV. Reducing external Mg^{2+} linearizes the current-voltage relation and increases both baseline current noise and NMDA-evoked currents while increasing external Mg^{2+} reduces noise and blocks NMDA currents.

Application of D-2-amino-7-phosphonoheptonoate (D-AP7), 7-chlorokynurenate, zinc or MK-801 had two effects: 1) Responses to NMDA were blocked and 2) Baseline current noise (especially in Mg^{2+}-free ringer) was reduced in amplitude. Responses to kainate were not affected by any of these agents.

Our results suggest that the external concentration of Mg^{2+} may play a role in controlling the extent to which NMDA receptors contribute to light-evoked responses in ganglion cells, especially near threshold.

Our data suggest that the NMDA receptor of the tiger salamander retinal ganglion cell possesses: a voltage-dependent Mg^{2+} block of the channel, a strychnine-insensitive glycine site, a zinc site and a phencyclidine/MK-801 site making these amphibian NMDA receptors similar to those in mammalian CNS neurons. (EY03014)

501.7

NEURAL CODING OF E-VECTOR AND COLOR IN GANGLION CELLS OF SALMONID FISHES. Craig W. Hawryshyn. Department of Biology, University of Victoria, P.O. Box 1700, Victoria, B.C., CANADA V8W 2Y2.

Studies have shown that fish can discriminate E-vector and orient to polarized light fields. However, these studies were conducted when little was known about ultraviolet (UV) sensitivity. Our recent studies have shown that UV-sensitive cones in addition to green- and red-sensitive cones play a major role in polarization sensitivity. The UV cones show a preference for vertical E-vector while the green and red cones both prefer the horizontal E-vector. This condition is necessary for discriminations on the basis of E-vector. Since the optic media of fish transmits UV radiation, a linearly polarized UV stimulus can stimulate all the cone mechanisms that overlap in the UV spectrum. Single-unit recordings have shown that UV/red opponent ganglion cells exhibit orthogonal polarization sensitivity. In one case, a UV-on/red-off unit was examined. When the UV polarized light stimulus was used under conditions of parallel sensitivity in the UV and red cones, a vertical E-vector orientation resulted in an on response, a horizontal orientation resulted in an off response and an oblique orientation resulted in an on-off response. Supported by NSERC(URF0043984).

501.8

HOW GANGLION CELLS SUMMATE EPSPS IN THE RECEPTIVE FIELD CENTER: PHYSIOLOGY, BIOPHYSICS AND COMPUTER SIMULATIONS. R.F. Miller, P.A. Coleman and S. Jayaram. Dept. of Physiology, Univ. of Minn., Minneapolis, MN 55455.

We studied the mechanisms by which ganglion cell dendrites summate EPSPs in the receptive field center of sustained On-ganglion cells in the mudpuppy retina. Our analysis was based on comparisons of whole-cell recording data (from the intact, perfused retina-eyecup), with computer simulations, using compartmental models derived from identified morphologies. Both linear and non-linear membrane were simulated for these studies, using our own software (NMA), combined with a commercially available analog simulator (Saber, Analogy Software). Bipolar cell inputs were simulated through conductance changes, whose time course was based on exponential (alpha) functions. Experimentally, we studied spatio-temporal summation in the receptive field center, using displaced spots and slits of light, whose intensity ranged from near threshold to well above threshold levels. We will present detailed models which account for the spatio-temporal summation properties of ganglion cell dendrites, for both large vs small signals and center vs distal inputs, to show that high values of membrane resistance (70,000 ohms cm^2) are required to adequately account for the physiology. Supported by NEI Grant EY-07376.

501.9

THE ROLE OF GANGLION CELL DENDRITIC ARCHITECTURE IN INCREASING THE SPATIAL BANDWIDTH OF CAT RETINAL W-CELLS. M.H. Rowe and J.F. Cox, Neurobiology Program, Ohio Univ., Athens, OH 45701.

The contrast sensitivity profiles of many cat retinal W-cells include a significant high frequency shoulder which makes it impossible for them to be fitted with a single gaussian function, and which indicates that the receptive field center mechanism itself is not gaussian in shape. We have used a model of the spatial sampling characteristics of ganglion cell dendrites to show that incomplete sampling of the underlying bipolar array by the ganglion cell could provide one possible explanation for this phenomenon. Given reasonable values for the size and spacing of bipolar cell receptive fields, it can be shown that incomplete sampling of the bipolar array within the domain of a ganglion cell dendritic tree will result in a sensitivity profile for the receptive field center which contains significant irregularities, i.e. a bumpy gaussian. The fourier transform of such a sensitivity profile produces a contrast sensitivity function which contains a high frequency shoulder very similar to those observed experimentally in W-cells, and this is true even when the irregularities are confined to the periphery of the dendritic tree. When applied to camera lucida drawings of HRP filled W-cells, the model produces 2 dimensional receptive field profiles which also contain significant irregularities, and whose fourier transforms include high frequency shoulders at some orientations. Thus, one consequence of incomplete sampling of the bipolar array is to extend the spatial bandwidth of the ganglion cell to frequencies about 30% higher than would be predicted from the overall dimensions of the receptive field. Supported by grants EY06013 and EY08038 from the National Eye Institute.

501.10

OSCILLATIONS IN CAT RETINAL GANGLION CELL RESPONSES TO LIGHT FLASHES. A.W. Przybyszewski, Dept. of Physiology, Freie Universität Berlin, Arnimallee 22, 1 Berlin 33, BDR.

The action potentials of ganglion cell (GC) responses to diffuse light flashes were convoluted with the second derivative of the Gauss function (Mexican hat) to obtain wavelet transformation. The width of the Mexican hat function was varied in 640 (OFF-center GCs) or 512 (ON-center GCs) steps (from 0.6 to 384 or 307ms), which gives different narrow band pass filters. This kind of analysis demonstrates a "mathematical" microscope, whereby the degree of magnification was varied. Some parts of the wavelet transformation were extended and analysed in the frequency domain to explore oscillatory components of the impulse pattern. The amount of different oscillations was richer in class I (Y) than class II (X) and in OFF- than in ON-center GCs. The range of analysed oscillations was between about 1000 and several Hz. Increase of the diffuse flash light intensity in the photopic range (0.5 to 100 cd m^{-2}) caused a shift in the frequency of some oscillations, e.g. in the primary activity period of ON-center GCs frequency increase proportionally to intensity. The change of the light intensity evoked new oscillations and changed the length of the old one. This method, by which the "local" (in time after stimulus) character of the different frequency components is preserved, could give insight into an interpretation of the neural circuity (our other abstract this Meeting).

501.11

AN ANALYSIS OF CAT GANGLION CELLS OSCILLATIONS BEFORE AND AFTER INTRAVITREOUS INJECTION OF AMINO-PHOSHONOBUTYRIC ACID (APB).

T.H. Chung*, O.-J. Grüsser and A.W. Przybyszewski. Dept. of Physiology, Freie Universität Berlin, Arnimallee 22, 1 Berlin 33, BDR (supported by ENA).

We compared the effects of APB injected (0.5-3mg pro eye) into the vitreous body on ganglion cell activity evoked by diffuse short (10ms) or longer (1s) light stimuli or eyeball deformation. The experiments were performed in pentobarbital anaesthetized cats. Single ganglion cell activity was recorded by means of microelectrodes from optic tract axon.

In on-center GC light-induced responses were completely inhibited one to two hours after APB injection. GC response to eyeball deformation was at first reduced and later completely disappeared significantly faster then the responses to light. This could be explained by the fact that APB also acts on synapses between horizontal and bipolar cells (Soc. Neurosci. Abstr.,15,part 1, p.925,1989). We used wavelet transformation (see our abstract this Meeting) to find oscillations in the response of GC to diffuse light flashes before and after APB injection. In some experiments the primary phase (maximum frequency of discharges) was strongly reduced in frequency after APB injection (from 1000-800Hz to 600-200Hz in 20-30min after APB injection). This reduction was not always synchronized with a disappearence of the GC's response to deformation. This supports the hypothesis that APB also acts on other synapses than photoreceptors - on bipolar cells as in lower vertebrates (Slaughter & Miller 81,86, Shiells & *al.*,81, Nawy & Copenhagen, 87).

501.12

COMPARING A RETINAL MOTION DETECTOR ALGORITHM TO CLASSICAL APPROACHES.

F.H. Eeckman, M.E. Colvin*, J.L. Teeters*, and T.S. Axelrod*. Lawrence Livermore Natl. Laboratory, Livermore, CA 94550.

Last year we presented a three-dimensional simulation of the motion detector circuitry in the vertebrate retina (Neurosci. Abstr. 366.23). The model consists of five layers of cells in a closely packed hexagonal array. All cells are modeled as single compartment, leaky-integrator neurons. The model-input consists of series of images of size 512 x 512.

We compare the performance of this model to some classical tracking approaches used to track dim targets against cluttered backgrounds. The test set consists of several 2d Gaussian blobs imbedded in independent and identically distributed Gaussian noise. The targets have a fixed signal to noise ratio with respect to the background and they move with different constant speeds. The algorithms we used for comparison are: adaptive thresholding and multi-stage hypothesis testing. Adaptive thresholding is computationally the simplest technique. Thresholding only works under conditions of constant contrast without contrast reversals, yet it is the most commonly used track detection method. Thresholding performance establishes a minimum performance limit against which all other algorithms can be judged. Multi-stage hypothesis (MHT) testing is the most complex technique. It starts a track at every pixel, in every direction, in every frame. It then uses statistical tests to weed out false tracks and to continue only those that are promising. In the Gaussian noise environment, MHT represents the upper limit of theoretically best possible performance. We found that the retinal algorithm is a powerful technique for target-extraction and we will discuss the importance of the various features of the biological algorithm in the realization of that performance.

SENSORY SYSTEMS—VISUAL CORTEX: RESPONSE PROPERTIES

502.1

RECEPTIVE FIELDS IN THE VISUAL CORTEX OF THE GRAY SQUIRREL:A DESCRIPTIVE STUDY.

F.W.GRASSO.UNIV. OF MASS.NEUROSCIENCE & BEHAVIOR PROGRAM,AMHERST,MA. 01003.

To broaden understanding of the comparative functions of visual-cortical neurons and move toward an ecologically inspired understanding of the neurophysiology of vision, maps were made of the receptive fields (RFs) of single units (n=55) in V1 of five Gray Squirrels(Scurius carolinensis). RF maps were made with an on-line computer system that presented a flashed or moving disc stimulus and collected responses in a manner previously used to map units in cats,monkeys and hamsters. The RFs could be classified by gross response properties as simple(65%),complex(22%), or unclassified(13%). They could also be classified by their spatial structure as Disc(40%),Bar (11%),Composite(22%),Diffuse(20%) or unclassified (8%). RFs classed Diffuse were always complex, Bar and Disc were always simple,while Composites were either complex or simple. The details of the spatial structure of the composite class vary across species. It is suggested that this class of RFs reflects species adaption to environmental niche. The maps will be compared to those made in other species.

502.2

SINGLE-UNIT ACTIVITY CORRESPONDING TO POPULATION BURSTS IN LAYER III OF RAT PRIMARY VISUAL CORTEX *IN VITRO*.

R. B. Langdon and M. Sur. Dept. of Brain and Cognitive Sciences, Massachusetts Institute of Technology, Cambridge, MA 02139.

In the rat primary visual cortex, single-shock stimulation to the base of a cortical column results in a brief burst of glutamatergically driven field potential spikes in layer III (Langdon and Sur, Soc. Neurosci. Abst., 1988, 1989, and *J. Neurophys.*, in press). We have proposed that these spikes represent synchronous firing of layer III output neurons linked by recurrent excitatory projections. We have now examined the relationship between single-unit recordings and these population events. Recording primarily from layer III, we have observed units that fire in brief bursts (2 to 6 spikes) in response to single-shock stimulations. As with the population bursts, spikes occurred 2.5 to 3.5 msec apart and amplitudes decayed rapidly. Post-stimulus latency histograms revealed individual spikes which matched the latencies of the "S1" (antidromic) and "S2" (synaptically driven) field potential spikes. Short latency spikes were more tightly phase-locked than later ones. Spikes occuring after 5 msec were prevented by 10 μM CNQX or low-Ca^{++} medium. In contrast, D-APV (50 μM) did not prevent short-latency (5 to 15 msec) spikes, but tended to reduce burst durations. In sum, layer III may contain a network of neurons that fire in bursts driven primarily by transmission through kainate/quisqualate glutamatergic receptors.

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502.3

SPATIAL AND TEMPORAL PROPERTIES OF CELLS IN THE RABBIT'S STRIATE CORTEX.

Casanova C.[1], Molotchnikoff S., McKinley P.A.[1], Morin C.*, Nault B.*, Michaud Y. [1]School of Physical and Occupational Therapy, McGill University, and University of Montreal, Montreal, Canada.

Bars and spots have been primarily used to determine the visual properties of cells in the rabbit's visual cortex. To our knowledge, drifting sinusoidal gratings have never been utilized to study the properties of single cortical cells despite the fact that gratings have been commonly presented to rabbits in discrimination tests (e.g. Van Hof et al., 1983). Tungsten-in-glass microelectrodes were used to record visual responses of cells in the striate cortex to drifting sinusoidal gratings of 80% contrast in New Zealand rabbits. A total of 35 cells were recorded in the visual cortex. Out of these, 26 units responded to drifting gratings, mostly with modulated discharges. More than half of the units were tuned to low spatial frequencies (SF) [mean= 0.16 c/deg; bandwidth < 2 octaves]. The remaining cells shown no attenuation of their responses to very low SF (low-pass cells). Out of these low-pass cells, two units were more responsive to a full-screen flicker rather than to the drift of a sine-wave grating. Almost half of the cells were tuned for a wide range of temporal frequencies (mean=3.6 +/- 2.6 Hz; bandwidth around 2.5 octaves), and the remaining cells were low or high-pass (6 and 2 units, respectively). Attempts have been made to record from all part of the visual field. So far, no differences have been noted between the cells' properties with respect to receptive field location (optic axis or binocular area of the visual field).

Supported by MRC, FCAR.

502.4

EVIDENCE OF LAGGED-TYPE GENICULATE INPUT TO VISUAL CORTEX.

A.B. Saul and A.L. Humphrey. Dept. of Neurobiology, Anatomy and Cell Science, U. of Pittsburgh, Pittsburgh PA 15261.

We previously characterized the response properties of lagged and non-lagged X and Y cells in the cat lateral geniculate nucleus (J. Neurophysiol., July 1990). Here, we are investigating their possible influence on visual cortex. Cortical simple cells were tested with narrow bars whose luminance was modulated sinusoidally at several temporal frequencies. First harmonic amplitude and phase were measured at a series of positions across the receptive field. Also, gratings were drifted in each direction at various spatial and temporal frequencies to characterize cells' directional tuning.

Lagged and non-lagged geniculate cells differ in response phase: lagged cells have a quarter-cycle phase lag and latencies 70ms longer on average. Cortical receptive field subzones similarly fall into groups separated by a quarter cycle phase lag and a 70ms latency increase. The phase difference between these two types of subzones is not as clear as that between lagged and non-lagged geniculate cells, but most simple cell subzones can be classified as lagged or non-lagged type according to their response phase. Some cells show purely lagged or non-lagged type subzones; other cells show both types of subzones.

The geniculate data suggested that lagged and non-lagged inputs to cortex might converge to produce direction-selective cells. From simulations, such cells would be expected to show direction selectivity only at low temporal frequencies, typically below 4Hz, because the inputs would lose their quarter-cycle phase difference beyond 4Hz and the non-lagged input would dominate at high temporal frequencies. Area 17 neurons often satisfy these predictions. The loss of direction selectivity at higher temporal frequencies can generally be predicted from the arrangement of response phase across the receptive field, as can the cell's spatial frequency tuning.

We conclude that lagged input from the LGN may be physiologically demonstrable in visual cortex and may contribute to direction selectivity, and spatial and temporal frequency tuning.

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502.5

EFFECTS OF NOREPINEPHRINE ON VELOCITY TUNING AND DIRECTION SELECTIVITY OF CAT VISUAL CORTICAL NEURONS. J. McLean, C-S. Lin, B.D. Waterhouse. Dept. Physiol. and Biophys., Hahnemann Univ., Phila., PA 19102

The effect of norepinephrine (NE) on the responses of striate cortical neurons to moving bars was studied using microiontophoretic techniques in anesthetized, paralyzed cats. Unit responses to optimally oriented bright and dark bars moving back and forth across the receptive field were measured at seven different speeds. Velocity tuning curves were then computed by taking the average maximum response for each speed, direction and bar contrast. A mean direction index was also calculated for responses to bright and dark bars. In the presence of NE (5-20nA) many of the cells examined demonstrated a shift in their velocity tuning curve such that the cell's preferred velocity was changed, while the shape of the velocity tuning curve was unaltered. In some cases the shape of the tuning curve was also modified during NE application such that the type of velocity response was changed, e.g. "velocity broad band" converted to "velocity low pass". In the majority of cells studied, the mean direction index increased in the presence of NE. For other cells, the velocity tuning and direction selectivity were unaffected by NE administration. Additional experiments on the same population of neurons revealed no obvious changes in orientation tuning. These results demonstrate that the spatiotemporal properties of striate cortical neurons are, at least, partially subject to modification by NE. In a broader context, these findings add a new dimension of specificity to the potential modulatory influence of the noradrenergic system on central processing of sensory signals. (Supported by AFOSR 87-0138 and an award from the Klingenstein Foundation to BDW)

502.6

INTRACELLULAR RECORDING STUDY OF STIMULUS SPECIFIC RESPONSE PROPERTIES IN THE CAT VISUAL CORTEX. SATO, H.[1], DAW, N.W.[2] and FOX, K.[2] [1]Dept. Neurophysiol., Biomed. Res. Ctr., Osaka Univ. Sch. Med., Kitaku, Osaka, 530 JAPAN, [2]Dept. Cell Biol. & Physiol., Washington Univ. Sch. Med., St. Louis, MO 63110, USA

Stimulus specific response properties were studied intracellularly in cells recorded from cat primary visual cortex. Animals were anesthetized with a gas mixture of 70 % N_2O and 30 % O_2 containing 0.5-0.7 % halothane and were paralyzed. In all five direction selective complex cells recorded, visually evoked EPSPs were tuned to one direction of stimulus motion (preferred direction), that is, EPSPs to the opposite direction were smaller than those to the preferred direction. Visually evoked IPSPs were also tuned to the preferred direction. These results suggest directionally tuned EPSPs make a major contribution to direction selectivity in complex cells. In an orientation selective simple cell, both EPSPs and IPSPs were tuned to an optimally oriented stimulus. A stimulus oriented diagonally to the optimal orientation evoked IPSPs but they were substantially smaller than those to an optimally oriented stimulus. We suggest the possibility that in the cat primary visual cortex excitatory inputs are tuned to optimal stimuli to produce direction/orientation specific responses and IPSPs evoked by non-optimal stimuli contribute to sharpening the direction/orientation tuning.

502.7

RESPONSIVENESS OF CELLS IN AREA 17 AFTER LOCAL INTERCEPTION OF THE DESCENDING PATH FROM AREA 18. B. Nault, Y. Michaud, C. Morin, C. Casanova, S. Molotchnikoff. Dept. Sciences biologiques, Université de Montréal, C.P. 6128, Succ. A, Montréal (Québec) Canada H3C 3J7.

There is no doubt that there exist massive reciprocal connections between visual cortical areas (17, 18, 19...). Thus, in addition to the predominant input from LGN, area 17 receives afferents which originate in area 18 and 19. In the present experiments, we have investigated the feedback path from area 18 to area 17. The strategy is to see the effect of local reversible inactivation of area 18, in the same retinotopic register as the site of the recording electrode in anesthetized cats.

To date 36 cells were fully tested. Data may be summarized in the following way: 63% of the cells were affected by the blockade and most cells saw their activity diminished. Interestingly some properties were more susceptible to the lack of input from area 18 than others. The average decrease in activity is 24% and we never observed a total loss of evoked discharges. It appears that the directionality is particularly sensitive to area 18 blockade.

In contrast to the feedforward influences (17 → 18), the present data suggest that area 18 has a modularoty effect on cells of area 17 by re-enforcing the input from the LGN.

Supp. MRC to SM.

502.8

THREE KINDS OF FUNCTIONAL COUPLING BETWEEN CAT AREAS A17 AND A18 REVEALED BY CROSS-CORRELATION.

J. I. Nelson[1], J. Bullier[2], P. A. Salin*[2], M.H.J. Munk*[1].
[1]Biophysics, Philipps University, Marburg, W. Germany 3550; [2]Exptl. Neuropsychology Lab, INSERM 94, Bron, France 69500.

Anatomical studies (Salin, Bullier, Kennedy JCN 1989, 283: 486) demonstrate that the connections between Areas 17 & 18 do not link only retinotopically corresponding regions. We have characterized these connections functionally by cross-correlating activity from pairs of microelectrodes in A17 & 18.

Sorted by width, the cross-correlograms (CCHs) fall into 3 non-overlapping populations (238 runs, 12 cats, 2 labs): towers (T, 2-9ms wide), castles (C, 10-70ms) & hills (H, 200-1000ms). T-, C- & H-couplings are independent; they occur separately or combined. Ts occur at 0 +/-1ms, suggesting common drive. Cs were also centered, but in a range of 5-15ms (1 area fires 1st); so offset Cs may arise from direct A17/A18 connections.

Ts are found only when receptive fields (RFs) have overlap (and usually similar orientations too); Cs are found equally often out of overlap (limit: 8 deg between RF centers). Without RF overlap, Cs occur only when preferred orientations match.

This functional coupling is sensitive to RF separation and orientation preference. Cs and esp. Hs possess the spatial and temporal spread needed to account for modulatory influences from outside the RF (Nelson & Frost, BR 1978, 139: 359; ExBR 1985, 61:54). Nevertheless, functional coupling without RF overlap, or matched orientations, or temporal delay requires the extension of current concepts of visual cortical organization.

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502.9

CHANGES IN RECEPTIVE FIELD (RF) SIZE OF SINGLE CELLS IN PRIMATE V1 AS A CORRELATE OF PERCEPTUAL COMPLETION. M. Fiorani Jr.*, R. Gattass, M.G.P. Rosa* and C.E. G.Rocha-Miranda*. Instituto de Biofisica, UFRJ, BRAZIL.

Dynamics of RF size of single cells in Cebus monkey V1 was studied in anesthetized and paralyzed preparations. Presentation of long bars revealed "RFs" driven by the contralateral eye, located within the projection of the blind spot (BS). Short bars within the BS elicit no response. Recording sites marked by electrolytic lesions were located within the representation of the BS revealed by CO histochemistry after monocular enucleation. This indicates that cells in V1 are capable of interpolating stimulation on the surround of a naturally blind region. To access the generality of this phenomenon we used opaque masks subtending 2 to 15 degrees to generate scotoma-like regions centered at eccentricities ranging from 5 to 20 degrees. Under masking conditions we observed three groups of cells: The first with no response; the second with extremely large RFs; the third had small RFs in the unmasked condition and continue to respond to large bars in the same location in spite of the use of masks. Thus, these cells respond at the "hidden RF" by interpolating over a large distance. The interpolated response were found at all tested eccentricities and present the same orientation and directional selectivity of those in the non masking condition. (Supported by FAPERJ, FINEP, CNPq and CEPEG-UFRJ).

502.10

LOCALIZATION OF UNITS IN V1 OF THE AWAKE MACAQUE *RESPONDING TO DIFFUSE ILLUMINATION*. T.M. Wengenack and S.J. Bolanowski, Dept. of Neurosurgery, Univ. of Rochester Med. Sch., Rochester, NY 14642 and Inst. for Sensory Research, Syracuse Univ., Syracuse, NY, 13244

We have found that approximately 51% of units (n= 736) recorded from V1 of the awake macaque respond to full-field (Ganzfeld) illumination (Wengenack, et al., SN Abst., 14, 1988). The response types fall into three categories: transient (18%), slowly adapting (13%), and sustained (i.e. luxotonic, 69%). Of those giving a sustained response, 72% could be binocularly activated and displayed one of four prominent response profiles: averaging (38%), inhibition (16%), summation (27%), and suppression (5%).

As determined on the anesthetized macaque, neurons in the cytochrome oxidase (CO) patches, as opposed to inter-patch regions, are typically non-orientation selective and may also respond to diffuse illumination. These two facts make it likely that the Ganzfeld-driven units described above reside within the CO patches. In an effort to verify this, we have electrolytically marked (elgiloy electrodes, Prussian Blue reaction) the various response types and compared their location to the CO patches. Surprisingly, there appears to be no relation between units responding to Ganzfeld illumination and the CO pattern. The units were found to occur both within CO patches and non-patch regions and they occurred in several different cortical layers. The results suggests that units responding to Ganzfeld stimulation for the awake animal can be orientation as well as non-orientation selective, and that anesthesia may affect the response properties of units in V1.

502.11

OSCILLATIONS OF ON-GOING AND EVOKED ACTIVITY IN NEURONAL ASSEMBLIES REVEALED BY REAL-TIME OPTICAL IMAGING IN CAT VISUAL CORTEX. A. Arieli, D. Shoham, R. Hildesheim* and A. Grinvald, The Weizmann Inst., Israel, IBM Res. Div. & Lab. of Neurobiol. The Rockefeller Univ.

In order to detect recurring patterns of activity in neuronal assemblies, we recorded, both optically and electrically, the spatio-temporal patterns of on-going and evoked activity of the same cortex. The visual cortices (areas 17 & 18) of anesthetized cats were stained with the dye RH-795. Cortical activity was evoked with stimuli consisting of drifting gratings. Optical signals (from 124 sites), Electroencephalogram (EEG), Local-Field-Potential (LFP) and spike activity (from two isolated neurons), were recorded simultaneously. Activity was recorded for 70 seconds in each trial.

We define "neuronal assembly" as a group of neurons that **cooperate** to perform a specific computational task. The activity of neurons in an assembly is **time-locked** (coherent). However, neurons belonging to different assemblies might not be spatially segregated. To separate the signals from different assemblies we used the fact that the activity of neurons in each assembly is coherent. Each spike was used as a trigger for averaging the optical signals. With sufficient averaging, activity of neuronal assemblies not containing the reference neuron was averaged out, enabling the **selective visualization** of the reference neuron's assembly.

This analysis revealed complex coherent patterns which "scanned" the cortex at several frequencies in the range of 3-50 Hz. Oscillations were detected at several frequencies mostly around 3 and 25 Hz. Surprisingly, analysis of the spatial patterns at different temporal frequencies revealed striking frequency dependent spatial structures of the neuronal activity. The spatial patterns of both on-going and evoked oscillations did not show a clear correspondence to the orientation columns. Interestingly we found that several recurring spatio-temporal patterns were very similar during both on-going and evoked activity. These results suggest that intrinsic on-going oscillations in neuronal assemblies play an important role in shaping spatio temporal patterns evoked by sensory stimuli.

502.12

COLOR FACILITATES MOTION CORRESPONDENCE IN VISUAL AREA MT K.R. Dobkins and T.D. Albright, The Salk Institute, La Jolla, CA 92037

A wealth of anatomical and physiological data suggests that motion and color are processed in separate channels in the primate visual system. While psychophysical data on this subject have been more equivocal, recent experiments have shown that color disambiguates motion correspondence in an apparent motion display (Green, Perc. and Psychophys., 45:15, 1989; Papathomas et al., ARVO Abs., 1989). We have used a similar paradigm to investigate the neural basis of chromatically facilitated motion perception. Our stimulus consists of alternating rows of isoluminant red and green dots (gaussian blurred) that are displaced by half of the inter-dot distance at 15Hz. Direction can only be disambiguated through color. Our human psychophysical data confirm that color facilitates motion and, furthermore, demonstrate that degree of facilitation varies with size of spatial displacement.

Neurons in cortical visual area MT are highly selective for direction of motion yet unselective for color. We recorded from single MT neurons in alert rhesus monkeys. An isoluminant red/green pair was found for each cell by the minimal response elicited by a drifting green bar varying in luminance on a red background. The apparent motion stimulus was then placed in the cell's receptive field. Responses to chromatic correspondence were compared in the preferred and null directions. Although direction selectivity was commonly weaker than that elicited by luminance correspondence, significant color-facilitated motion correspondence was seen for the majority of cells. Degree of facilitation varied as a function of spatial displacement.

These results confirm the presence of a functional chromatic input to the magno pathway and imply true chromatic "gating" of directional selectivity. Psychophysical experiments suggest that color may be but one of many low-level cues (e.g. orientation) that facilitate motion correspondence. It remains to be seen whether cue facilitation is a general phenomenon underlying direction selectivity in MT.

502.13

ANALYSIS OF RELATIVE MOVEMENT BETWEEN AN OBJECT AND ITS BACKGROUND IN THE MACAQUE VENTRAL MST AREA.
Y. Sugita and K.Tanaka Lab. for Neural Information Processing, RIKEN, Wako, Saitama 351-01, Japan.

Cells in the ventral MST area responded to a small-field movement with directional selectivity. They did not respond to a wide-field movement, but when a stationary object was placed in front of the moving background, about 40% of them responded in the direction opposite to their preference for the small-field movement. We examined the properties of this reversed response to the background movement as well as the mechanisms of the response, by using anesthetized (N_2O) and immobilized (gallamine triethiodide) monkeys (M. fuscata). These responses are not specifically related to pursuit eye movements, because (1)their preferred speed for the object movement roughly corresponded to that for the background movement, and (2)the reversed response was evoked as long as the object was placed within the receptive field, and, furthermore, the receptive field did not necessarily include the fovea. Instead, they are likely to be involved in the general analysis of the object motion relative to its background. The reversed response was considerably reduced by decreasing the background diameter from 80 to 40 deg. The response was abolished by blurring the border of the object, whereas a slight change in object size did not decrease the response. We conclude that not only movement of the wide-field background but also phenomena at the border of the object are necessary to evoke the response.

502.14

DISTRIBUTION OF RESPONSE PROPERTIES ACROSS TOPOGRAPHIC SUBDIVISIONS OF MACAQUE PRELUNATE GYRUS. M. Youakim* and J.S. Baizer Dept. of Physiology, University at Buffalo, Schl. Med., Buffalo, NY 14226.

We have studied response properties and topographic organization of the prelunate gyrus in four hemispheres of two awake, behaving, pigtail macaque monkeys. Topography in the pigtail macaque is similar to that reported for the rhesus (Maguire and Baizer, 1984). A vertical meridian representation (VM) ran diagonally across the gyrus, 10-13 mm lateral to the junction of the lunate and intraparietal sulci. Anterior and lateral to the VM was V4 (Area AL of Maguire and Baizer). Posterior and medial to it was Area PM. We compared single cells in V4 and PM for sensitivity to several stimulus dimensions, including orientation, color, size, and effect of background stimuli. Two response properties, orientation sensitivity and background modulation, distinguished PM from V4. Area PM contained a significantly greater proportion of orientation sensitive cells (73%, 47/64) than V4 (35%, 30/86, $p<.001$). In V4, however, a greater proportion of cells (37%, 15/41) showed dramatically enhanced responses to a visual stimulus in the presence of a background pattern than in PM (5%, 1/20, $p<.05$). Supported by MH42130 and the Whitehall Foundation.

502.15

STIMULUS SELECTIVITY OF INFEROTEMPORAL CORTEX NEURONS: SIMULTANEOUS RECORDING FROM ADJACENT CELLS. I. Fujita, K. Cheng* and K. Tanaka. Lab. for Neural Information Processing, RIKEN, Wako, Saitama 351-01, Japan.

The anterior part of the inferotemporal cortex (AIT) of the monkey contains cells which selectively respond to particular visual patterns. An essential step towards understanding how visual pattern information is processed in AIT is to learn how cells with different selectivities are spatially arranged. This study addressed the question of whether neighboring AIT cells have similar selectivity for visual pattern stimuli.

A monkey (*Macaca fuscata*) was anesthetized with a mixture of N_2O and O_2 and was immobilized with gallamine triethiodide during the experiments. We recorded simultaneously from several adjacent AIT cells with a single electrode. The electrode position was adjusted so that activity of one cell could be reliably isolated from multiple unit activity with a threshold discriminator. Responses of the isolated cell were tested with a set of stimuli including tens of 3-D objects. With the aid of a computer graphics system we then determined what aspects of the effective stimuli were essential to activate the cell. Throughout these procedures, peristimulus time histograms were generated for the isolated cell and for nearby cells, which were selected by a second window discriminator.

Adjacent cells showed similar or related selectivity in most cases. They were selective to limited and overlapped ranges of the stimulus feature spectrum, although the degree of selectivity or the optimal features were slightly different among adjacent cells. For instance, one cell responded to a "reversed T" pattern (⊥), but not to a "reversed Y" (人) or an arrow (↓), and a cell simultaneously recorded responded to all 3 stimuli. Neither cell responded to other stimuli including a "T", an "L", a "mirrored L" (⅃) and bars (_ or |). Crosscorrelograms of simultaneously recorded cells showed either symmetrical or asymmetrical peaks indicating that the cells were functionally connected. Clustering of cells with similar selectivity and functional connections suggests that local interactions among neighboring AIT cells contribute to selectivity to visual pattern stimuli.

502.16

DERIVATION OF POPULATION VECTORS FROM THE RESPONSE SPECIFICITIES OF VISUAL CORTICAL CELLS.
M.P. Young* and K. Tanaka. Lab. for Neural Information Processing, RIKEN, Wako-shi, Saitama, Japan.

It has often been suggested that the cell population may be a useful level at which to analyse activity in neural systems. Population vectors have been derived for motor cortical cells by assuming that cell preferences may be defined in the vector space in which movements are made (Georgopoulos A.P., et al, Science, 233:1416-9, 1986). Population vectors could also be derived for visual cells with simple stimulus specificities by an analogous assumption about the dimensions of the relevant space. Many visual cells, however, have more complex 'trigger' features. The first step in the derivation of population vectors for these more complex cells is to find a Euclidean space in which the response properties of each cell may be represented. This can be accomplished by applying multidimensional scaling (Shepard, R.N., Science, 210:390-398, 1980) to a table of the responses of cells to a number of stimuli. The stimulus preference of each cell may then be represented in the derived space as a vector, or, to preserve information about the cell's breadth of tuning, as a statistical distribution. Population vectors or population distributions can then be derived by summation after weighting the cell vectors by response magnitude.

This approach can be applied to the responses of local populations of anterior inferotemporal (AIT) cells which respond to a narrow range of complex stimuli. A feature of these local populations is that neighbouring cells have similar stimulus specificities (see Fujita, I., et al., this meeting). This populational analysis can be used to determine what stimulus feature is signalled by a particular local population of cells. Population distributions, derived in this way, allow comparison between the properties of cell populations and psychophysical representations of the discriminability of stimuli, when these are given in terms of signal detection theory.

502.17

VISUAL AND OCULOMOTOR PROPERTIES OF SINGLE NEURONS IN POSTERIOR CINGULATE CORTEX OF RHESUS MONKEY. S. Y. Musil, C. R. Olson, and M. E. Goldberg. Laboratory of Sensorimotor Research, National Eye Institute, Bethesda, MD 20892, and Dept. of Psychology, George Mason University, Fairfax, VA 22030.

The posterior cingulate cortex (CGp) of the rhesus monkey is widely regarded as a paralimbic area with functions related to emotion and motivation. However, it is linked by a strong reciprocal pathway to parietal Area 7a which participates in visual, attentional and oculomotor processes. To see if CGp is involved in these processes we recorded from single neurons in CGp in a rhesus monkey trained to perform a variety of visually guided oculomotor tasks.

Neuronal activity was modulated in relation to task performance in roughly half of the neurons forming our sample. The factors most strongly influencing the level of activation were the orbital position of the eye and the amplitude and direction of saccades. Neurons influenced by orbital position characteristically discharged tonically when the eye was in an extreme position and exhibited a graded decrease of excitation as the eye deviated from this position. Neurons influenced by saccades changed their firing level at or shortly after saccade onset. They were broadly tuned for direction and fired most strongly during and after large saccades. Although initial and final orbital position affected the response of these postsaccadic neurons, they did not discharge when a seemingly effective locus was acquired by an ineffective direction or amplitude of saccade. These neurons did not respond to small (1^0) spots of light, but because even saccade-related activity was often modulated by background illumination they must be visually responsive.

These results suggest that CGp neurons participate in visuomotor or visuospatial functions depending upon accurate knowledge of eye position and recent eye movements. They do not support a uniquely emotional or motivational function for CGp.

502.18

MODALITY SPECIFICITY OF NEURONAL RESPONSES IN THE ANTERIOR ECTOSYLVIAN CORTEX (AEC) OF CATS. H. Jiang*, F. Lepore, M. Ptito and J.-P. Guillemot. Groupe de Rech. en Neuropsy. Exp., Université de Montréal and Université du Québec, Montreal, Qué., Can. H3C 3J7.

In order to establish the modality specificity of the AEC and to determine whether it is a polysensory area, extracellular recordings were carried out along the banks of the Anterior Ectosylvian Sulcus (AES) in anesthetized-curarized cats. Three types of stimuli were used: visual stimuli consisted of light or black bars presented at optimal speed and orientation; air puffs and light touches applied to different body regions made up the somatosensory stimuli; auditory stimuli were presented through a speaker positioned either in front or on either side of the interaural line on the horizontal plane. Results showed that unimodal, bimodal (visual-auditory, visual-somatosensory, auditory-somatosensory), and trimodal units were distributed about equally in the AES, with a slight predominance of the latter type of units. Thus, approximately 2/3 of the units responded to at least two modalities. The receptive fields (RFs) of cells with visual or/and somatosensory modalities were almost always large and bilateral. The somatosensory RFs covered either the face, whiskers, head, or/and neck, the trunk or/and limbs and the tail. The response to stimulation in one modality in a multimodal unit was not always equal to that evoked in the same unit to stimulation of a different modality. There were no significant differences in the modality distribution along both the ventral and dorsal surfaces of the sulcus. The high proportion of multimodal units suggests that AEC is a polymodal area which is probably involved in integrating sensory input with action.

502.19

RETINOTOPIC ORGANIZATION AND RECEPTIVE FIELD CHARACTERISTICS OF NEURONS IN THE VISUAL WULST OF THE PIGEON. E. Sproule* and B.J. Frost. Department of Psychology, Queen's University, Kingston, Ontario, K7L 3N6, Canada.

In the avian visual system, a significant number of retinofugal fibres project to the dorsal thalamic nucleus complex (n. opticus principalis thalami, or OPT). The main ascending target of the OPT is a laminated structure on the dorsal surface of the telencephalon known as the Wulst.

Although there are reports in the literature which indicate the presence of retinotopicity in the pigeon Wulst, the retinotopic organization of this structure has not been systematically investigated. We used standard electrophysiological techniques to record from single units in the Wulst, and mapped their receptive field positions. In addition, we measured the sharpness of tuning of units selective for the orientation of bar stimuli.

We mapped the contralateral visual field within the Wulst, and observed a heavy overrepresentation of the region of binocular overlap. Thirty-four percent of the isolated units were orientation-selective and appeared to be more broadly tuned than neurons in mammalian striate cortex. The data are consistent with previous suggestions that the visual Wulst may be functionally analagous to mammalian striate cortex.

CORTEX V

503.1

NEURONES IN PRIMATE TONGUE MOTOR CORTEX ALTER THEIR FIRING RATES DURING SWALLOWING. G.M Murray+ and B.J. Sessle. Faculties of Dentistry, Univ. of Toronto, Canada M5G 1G6, and +Univ. of Sydney, Australia 2006.

Our recent studies have shown that many neurones in tongue motor cortex (tongue MI) alter their firing rates during a tongue-protrusion task performed by a monkey. This study's aim was to see if tongue MI neurones also alter their firing rates during semi-automatic orofacial movements such as swallowing. Extracellular single neurone recordings were made from tongue MI (defined by intracortical microstimulation; $\leq$20 μA) in an awake monkey (M. fascicularis) swallowing the fruit juice reward associated with successful performance of tongue-protrusion task trials; EMG recordings were also made from the genioglossus muscle. For each of 76 tongue MI neurones, the firing rates during the task and during the period of genioglossus EMG activation associated with the swallow were compared statistically (ANOVA) with that during a preceding control period. The neurones were found to be (i) swallow-related only (14%, 11/76), i.e., each neurone exhibited significant changes (vs. control) in firing rate during swallowing only, (ii) both swallow- and task-related (33%, 25/76), or (iii) unrelated to swallowing and the task (20%, 15/76). These data suggest that tongue MI may be involved in the execution of orofacial movements associated with swallowing.

Supported by Canadian MRC.

503.2

SOMATOSENSORY AND MOTOR CORTICAL REPRESENTATIONS OF THE TONGUE AND THEIR CORTICOFUGAL MODULATION OF THE JAW-OPENING REFLEX (JOR) IN RATS. C.Y. Chiang, J.O. Dostrovsky and B.J. Sessle. Dept. of Physiology, Fac. of Medicine, and Fac. of Dentistry, Univ. of Toronto, Toronto, Ont. M5S 1A8, Canada.

The aim of this study was to compare the effects of stimulation of tongue somatosensory and motor cortices on the JOR. Cortical evoked potentials (EPs) produced by lingual nerve (Lg) stimulation were recorded in chloralose/ketamine-anaesthetized rats and were located in a sagittally oriented strip of cortex (2x4 mm) approximately 6 mm lateral to Bregma. The largest EPs (2-2.5mV; latency 5.2$\pm$0.2ms, n=11) were recorded near the rostral end of this strip (A2-2.5, L6-7 with respect to Bregma). The tongue motor area (1.5x2.5mm) was mapped with intracortical microstimulation (ICMS;<60uA, 10ms train of 0.2ms, 300Hz pulses); lowest threshold ICMS sites (mean 30.7$\pm$4.2 uA, n=13) were located at A1-2, L6-7. Although the tongue motor area overlapped extensively with the somatosensory area (as defined by EPs) an effective ICMS site could be found where there was no clear Lg-induced EP. Conditioning stimulation (10ms train, 50uA) at this motor area site produced a pronounced facilitation of the JOR induced by Lg test stimuli (>200% of control JOR at 11-15ms) followed by a 200ms inhibitory phase that was maximal (25% of control) at a conditioning-test interval of 50ms. In contrast, similar conditioning stimulation at the somatosensory site of maximal Lg-induced EPs produced similar but smaller facilitatory (130%) and inhibitory (40%) effects on the Lg-induced JOR. These findings indicate that tongue sensori-motor cortex has both facilitatory and inhibitory actions on the JOR and that the tongue motor cortex may exert more powerful modulatory influences than tongue somatosensory area on the Lg-induced JOR.

503.3

MODULATION OF RESPONSES OF PRIMATE FACE SOMATOSENSORY CORTEX SI NEURONES TO PERIPHERAL STIMULI DURING TRAINED MOTOR TASKS. L.-D. Lin, G.M. Murray, and B.J. Sessle
Fac. Dentistry, Univ. of Toronto, Toronto, Ont., M5G 1G6

Modulation of somatosensory transmission in limb SI neurones has been implicated in limb motor control. This study's aim was to see if somatosensory transmission in face SI neurones is modulated in relation to orofacial movements. Extracellular single neurone recordings were made in 2 awake monkeys (M. fascicularis) trained in tongue protrusion and biting tasks. Mechanical (200 ms) or electrical (100 μs) stimuli were applied to the mechanoreceptive field (RF) of single neurones with a perioral RF, or the lingual nerve was electrically stimulated for neurones with a tongue RF. Evoked responses of neurones were tested during, and for 1-s prior to, the movement associated with each task. Depression of evoked activity occurred in only 10 of 19 neurones tested during the biting task but was noted in all 16 neurones tested during the tongue task; modulation of the evoked activity often occurred 50-100 ms before the onset of tongue EMG activity associated with the tongue task. No modulation was noted prior to or during the tasks for 3 neurones tested in limb SI that had a RF on the hand. These data suggest that somatosensory transmission in SI neurones may be modulated prior to and during voluntary orofacial movements; the presence of modulation may depend on RF location and the nature of the task. Supported by Can. MRC.

503.4

DISTRIBUTION AND PROPERTY OF THE ORO-FACIAL SI NEURONS IN RELATION TO MASTICATION IN AWAKE CATS. H. Hiraba, T. Yoshida*, C. Tsujimoto* and R. Sumino*. Dept. of Physiol., Sch. of Dent., Nihon Univ., 1-8-13 Kanda Surugadai, Chiyoda-ku, Tokyo 101, JAPAN.

Single neuronal activities were recorded in the oro-facial area of the first somatosensory cortex (the oro-facial SI) of 8 awake cats, to investigate the functional role of sensory information during mastication. Fifty-nine percent of the recorded oro-facial SI neurons (365/620) showed a rhythmical burst firing in relation to the movements of the jaw and the tongue during mastication. Approximately 75% of the mastication related (MR) neurons were found in the perioral and the tongue projection areas of the oro-facial SI. These MR neurons had receptive fields in the perioral region, tongue and teeth which were easy to be stimulated during mastication. However, distinction between MR and non-MR neurons with the same receptive field depended on differences in the direction-sensitivity and threshold value to stimulation during mastication.

These results suggest that the MR neurons would supply sensory informations required the performance of mastication.

We investigated the neuronal activity in the motor cortex (MI) in relation to mastication. We will also discuss to be different in the neuronal activity between the SI and the MI.

503.5

MULTIPLE CONTROL OF CEREBRAL CORTEX IN EYE MOVEMENTS. Y. TAMAI and A. KIMURA, Dept. of Physiology, Wakayama Medical College, Wakayama 640, Japan

Neuron discharges in the eye movement-evoking cortices (EMECs) were studied in relation to visually or auditory guided saccades of the cat. Experiments were carried out using nonanesthetized cats with chronic microelectrodes implanted simultaneously in the EMECs: the medial wall of the hemisphere under the cruciate sulcus, the medial and lateral bank of the presylvian sulcus, the fundus of the coronal sulcus and the ventral bank of the anterior ectosylvian sulcus. The cat was sitting in the box and moving her eyes according to the light or sound signals. There were two types of neuron in the EMECs, the one was locked to the sensory signal and the other was locked to the eye movement. The movement-locked neurons, however, were not always active in an specific EMEC but discharged simultaneously or alternatively between the EMECs. These results suggest that the eye movement may be controlled by many cortices located in the different portion of the corebral cortex.

503.6

DISTRIBUTION OF MOVEMENT RELATED POTENTIALS (MRP) IN A MONKEY: EFFECTS OF A UNILATERAL LESION OF THE SUPPLEMENTARY MOTOR CORTEX. N.S. Nicholson and J.C. Arezzo, Albert Einstein College of Medicine, Bronx, New York 10461

Identification of the neural generators of MRPs requires topographic mapping of individual components. For this purpose a macaque was implanted with 104 epidural electrodes extending bilaterally from the principal to the lunate sulci. The animal was trained to perform self-paced, right wrist movements; EMG was used to trigger MRP averaging (1 s epoch with 500 ms pre-movement). Five MRP components were identified; 3 were mapped in detail. N2A and N2B are phasic negativities which each begin less than 325 ms before the EMG burst and peak at approximately 70 ms prior to, and coincident with, the EMG, respectively. P2B is a large positivity which follows EMG onset. The N2A component is maximal overlying the contralateral motor (MI) and supplementary motor areas (SMA), with a significant distribution overlying equivalent ipsilateral regions. N2B is also distributed bilaterally, centered over MI although less prominent ipsilaterally. P2B is characterized by a broad contralateral distribution centered over the somatosensory region.

Following initial mapping, the animal suffered an accidental punctate lesion of the left SMA, portions of the basal ganglia and the anterior commissure were also involved. Clinical sequelae included focal motor seizures followed by temporary right upper extremity paralysis. After functional recovery the contralateral EMG was indistinguishable from pre-lesion studies. N2A, however, was significantly reduced in amplitude ($P<0.01$) and its distribution was restricted to the posterior contralateral MI. N2B showed similar but less severe changes (approximately 65% decrease contralaterally). P2B showed little overall change in amplitude or distribution.

These findings suggest that SMA participates bilaterally in the initiation of movement as represented by the phasic negativities seen immediately prior to and coincident with the EMG burst. Furthermore, they confirm the sensitivity of surface MRPs to subclinical cortical motor dysfunction.

503.7

DEFICIT IN UNIMODAL (TACTILE) AND CROSSMODAL DELAYED MATCHING FROM COOLING PREFRONTAL CORTEX. J.M. Fuster, B.V. DiMattia*, K.A. Posley* and W.W. Shindy*. Department of Psychiatry and Brain Research Institute, UCLA School of Medicine, Los Angeles, CA 90024.

The purpose of this research was twofold: (A) to acquire normative data on the ability of monkeys to learn a haptic (tactile) memory task (delayed matching-to-sample); and (B) to examine the effects of reversible cryogenic lesions of dorsolateral prefrontal cortex on performance of the task. A task-trial essentially consisted of the following sequence: (1) palpation or visualization of a stereometric object (the sample); (2) delay (5-60 sec); and (3) presentation of two objects for a haptic or visual choice--rewarded if correct (i.e., if the chosen object matched the sample object). Three monkeys were trained to perform the task with several pairs of objects differing in shape, size, or texture. Learning rate and efficiency varied among animals. One animal did not learn to make haptic choices. The other two learned to perform the task faster and to a higher level in the visual-haptic mode (visual sample, haptic choice) than in the other two modes (haptic-visual and haptic-haptic). In all three animals, learning rate and final level of performance depended on the object pair and the delay. Performance decreased as a function of delay. After training, cooling probes were implanted--under general anesthesia--on dorsolateral prefrontal cortex and posterior parietal cortex in the two animals that learned the task in all three modes (visual-haptic, haptic-visual, and haptic-haptic). Bilateral prefrontal cooling to 15° C induced a reversible performance deficit in all three modes. No significant performance deficit was induced by bilateral parietal cooling (15° C) on haptic-haptic performance. The results indicate a deficit in tactual as well as visual short-term memory from functional depression of dorsolateral prefrontal cortex. This deficit obtains for the three modes of cross-temporal integration performed by the animals in these experiments.

503.8

PREFRONTAL AND POSTERIOR PARIETAL UNITS IN CROSS-TEMPORAL VISUO-MOTOR (COLOR-DIRECTION) ASSOCIATION. J. Quintana and J.M. Fuster. Department of Psychiatry and Brain Research Institute, UCLA School of Medicine, Los Angeles, CA 90024.

The extracellular activity of 152 posterior parietal units was recorded and compared with that of 204 dorsolateral prefrontal units in a monkey performing a visuo-motor task. The stimuli and responses were dissociated in space and time. A central color stimulus (1) was followed by a 12-sec delay and by a second stimulus (2)--a pair of color or white lights--prompting a manual response to either the right or the left side, according to the following contingencies:

Stimulus 1	Delay	Stimulus 2	Response direction
Yellow or Green	12 s	White lights	Right
Blue or Red	12 s	White lights	Left
Red or Green	12 s	Red and Green	Matching color (random side)

Units were classified according to their differentiation of stimulus color and/or response direction. Color-coupled units were about equally numerous in both cortices (10.3% in prefrontal; 12.5% in parietal). On the average, the per bin ratio postcue/baseline of the activity of those units in trials with the preferred color decreased during the delay. Direction-coupled units were more common in parietal than prefrontal cortex (27.6% vs 18.1%). Of these, units that showed direction related activity anticipating the correct response position throughout the delay were twice more common in prefrontal cortex (7.8% vs 3.9%). Such units showed, on the average, a gradual increase of delay activity after the cue. The slope of this increase seemed to be a function of the probability of the future response direction associated with each color. Units with direction related activity restricted to the choice period showed larger reactions in parietal than prefrontal cortex. Some units in both cortices showed activity related to the predictability (i.e., certainty or uncertainty) of the response direction. These results suggest that neurons in dorsolateral prefrontal and posterior parietal cortices may be part of distributed cortical networks for visuomotor cognitive processing. Prefrontal units seem especially involved in the preparatory aspects of the impending action and posterior parietal units in the spatial aspects of the action at the time of its occurrence.

503.9

THE PREFRONTAL CORTEX AND SENSORIMOTOR BEHAVJOR: EFFECTS OF HORMONAL AND CIRCADIAN CYCLES. A.G.C. Bergqvist, M.C. Kennedy and S.S. Smith, Dept. of Anatomy, Hahnemann Univ., Philadelphia, PA 19102.

Neurons from the rat dorsomedial prefrontal cortex (PFC), the frontal pole and mediodorsal (DM) thalamus were recorded simultaneously using chronically implanted arrays of 5-7 microwires (0.001" dia). Implanted animals were trained to locomote on a computer- controlled treadmill device (alternately on-off, at 10 s intervals) for a period of 1-2 hs daily during neuronal recording. Cross-correlated activity was recorded from the PFC and its DM thalamic afferents in order to define a functional connectivity and establish the CNS site within this network relevant to behavioral and hormonal effects on the system. In addition, in order to investigate possible learning effects related to PFC activity and hormonal states, a randomly generated tone-signalled treadmill onset was implemented in the behavioral paradigms. PFC activity was found to be correlated to a greater degree with locomotion (tread on) compared with non- movement (tread off) when neurons in this area were recorded from rats on diestrus 1 or 2 (D), a period of relatively low estradiol levels, during the light segment of the light:dark cycle. The tread off- correlated discharge was markedly augmented by three- fold on estrus (E), following the cyclic increase in circulating, endogenous estradiol, relative to values obtained on D. In many cases, cells exhibiting tread on-correlated maximal activity were transformed to tread- off-correlated cells. When tested during the dark part of the cycle, tread-off correlated discharge was higher on E when compared with similar values during the light. Furthermore, increasing treadmill speed (from 4 to 11 cm/s) produced greater alterations in neuronal-correlated discharge when tested on D compared to E. Both estrous cycle and circadian effects on neuronal responses to tone-signalled treadmill onset were also observed. Thus, the present results suggest that hormonal as well as light-dark fluctuations produce alterations in the response of PFC neurons to pre-movement conditions. These results are also consistent with the hypothesis that the PFC may play a role in spatial memory through processing of temporal information. In addition, the PFC may be a relevant CNS locus for the reported effects of estradiol on sensorimotor activity and certain visuospatial learning tasks. *Supported by NS 25809 to SSS.*

503.10

TOPOGRAPHIC REPRESENTATION OF MEMORY IN THE PREFRONTAL CORTEX OF MONKEYS REVEALED BY LOCAL APPLICATION OF BICUCULLINE. T. Sawaguchi and P.S. Goldman-Rakic. Sec. of Neuroanatomy, Yale Univ. Sch. of Med., New Haven, CT 06510.

To examine the functional organization of the prefrontal cortex for spatial working memory, bicuculline was locally injected into the dorsolateral prefrontal cortex while monkeys performed an oculomotor delayed response task. The monkeys fixated a central spot and made memory-guided saccades to a visuospatial target which had been presented prior to a brief (usually 5 sec) delay period. In each trial the target was presented at one of several (> 22) peripheral locations with different eccentricities and/or directions. The local injection of bicuculline (1-10 ug/ul) induced deficits in the memory-guided saccade to a few specific target locations that varied by the injection site but usually contralateral to it. The deficit was characterized by increases in the variability and discrepancy between target and memory-guided saccades made to it. The deficit was sensitive to the duration of delay, and longer delays were associated with larger deficits. Furthermore, the relationship between the affected location of the target and injection sites showed a topographic manner: more peripheral locations were affected by injections into more lateral regions, and upper locations were affected by injections into more caudal regions of the dorsal prefrontal cortex. No deficits appeared in a control task given in the same session which required only sensory-guided saccades. The results suggest that memory modules for specific visuospatial representations are topographically represented on the prefrontal cortex.

503.11

DELAY- PERIOD ACTIVITY OF PREFRONTAL NEURONS IN DELAYED SACCADE AND ANTI-SACCADE TASKS. S. Funahashi and P.S. Goldman-Rakic, Sect. of Neuroanatomy, Yale Univ. Sch. of Med., New Haven, CT 06510.

The directional delay-period activity of prefrontal neurons is thought to reflect a mnemonic event, holding "on line" information that will guide the correct response at the end of the delay. In the present study we hoped to learn more about the nature of the mnemonic code; specifically, whether it reflects information about the coordinates of the sensory cue or the direction of the impending response. To examine this issue, we compared neuronal activity under two task conditions: a delayed saccade task (DS), in which the correct saccade was always to the same direction signaled by the cue, and a delayed anti-saccade task (DAS), in which the required saccade was to the direction opposite that signaled by the cue.

A total of 44 neurons with directional delay-period activity in the DS task was examined in the DAS task. The majority (n=30) showed the same directional delay-period activity, i.e., responded only when the cue was presented at the same location in both tasks, regardless of the direction of the saccade performed. The specificity for cue direction in these neurons was further illustrated by their pattern of activity on DAS trials in which the monkey failed to inhibit the prepotent response tendency. On these trials, which are identical to correct trials in the DS task, the neuron also discharged in accord with the location of the cue. Ten additional neurons showed delay-period activity which depended on the direction of the forthcoming saccade and the remaining 4 neurons exhibited directional delay-period activity only in the DS task.

The present results with respect to oculomotor responses are in accord with data originally obtained for manual delayed-response tasks by Niki and Watanabe (Brain Res,105:79,'76). Both studies indicate that independent prefrontal neurons code cue and response direction and that each is a distinct component of the circuitry involved in memory-guided responding. We speculate that the smaller proportion of neurons that code saccade direction are closely linked to structures which generate saccades, whereas the larger fraction of neurons that maintain a representation of sensory events could supply directional information to be read out by any of several motor centers. The independence of sensory-coded and response-coded memoranda provides a mechanism for flexible response choice. Supported by MH44866, MH38546.

503.12

COMPUTER STUDIES OF THE ROLE OF NMDA RECEPTORS AND POSITIVE FEEDBACK LOOPS IN THE GENERATION OF DESCENDING MOTOR COMMANDS. L. N. Eisenman, J. Keifer and J. C. Houk. Dept. of Physiology, Northwestern Univ. Medical Center, Chicago, IL 60611.

Positive feedback in recurrent loops between the cerebellum, red nucleus and motor cortex is thought to be important in the generation of central motor commands (J.C. Houk *In*, Models of Brain Function, p. 309, 1989). A computer simulation of cerebellorubral elements has been implemented to test this theory. Positive feedback between reciprocally connected neurons was triggered by a transient sensory input and regulated by preset levels of inhibitory input from cerebellar Purkinje cells.

The activation function for a neuron was motivated by physiological data derived from the *in vitro* turtle preparation, which suggests that motor commands in the red nucleus are produced by recurrent excitation from the cerebellorubral circuit mediated by combined NMDA and non-NMDA dependent synaptic transmission. The voltage dependency of the NMDA-associated channels was modeled as a threshold-like discontinuity in the activation function assumed for the model neurons. When a pair of cells with these properties were reciprocally connected, two stable regions resulted. There was an off state and an on state that had a wide range of stable operating points. The intensity of activity in the on state could be modulated by inhibitory input representing the effect of Purkinje cells. In contrast, when activation functions without discontinuities were used, the on state of the network was much less controllable.

Twelve reciprocally connected two-cell pairs were interconnected to model divergence in linkages between the motor cortex and the interpositus nucleus of the cerebellum. Each of the 12 elements is considered to have a preferred direction sensitivity. With this network several observations of Georgopoulos and colleagues regarding population vectors in motor cortex were reproduced. The values of the inhibitory input determined the final state of the network which reproduced activity patterns that resembled population vectors. It was further possible to simulate rotations of population vectors similar to those which have been related to mental rotation of images (Georgopoulos et al., Science 243: 234, 1989).

503.13

NETWORK MODEL OF THE CEREBELLUM AND MOTOR CORTEX THAT LEARNS TO CONTROL PLANAR LIMB MOVEMENTS. A.G. Barto, N.E. Berthier, S.P. Singh* and J.C. Houk, Dept. of Computer and Information Sciences, Univ. of Mass., Amherst, MA 01003. Recently we proposed a new model that treats the cerebellum as an array of modules that function as adjustable pattern generators (Houk et al, *Neural Networks for Control*, Miller, Sutton & Werbos, 1990). Here we report on the capabilities of small arrays of these modules for learning to control two-dimensional movements. The present modules are inspired by interconnections between the cerebellum and the motor cortex and the goal was to control a set of planar limb movements in a task analogous to the one used by Georgopoulos and coworkers to study populations of motor cortical neurons. The synapses between parallel fibers and Purkinje cells (PCs) were adjusted using training signals transmitted by climbing fiber inputs and a training rule inspired by the biochemistry of intracellular messengers regulating LTD at parallel fiber synapses. Climbing fibers were assumed to fire during corrective movements with a probability that was broadly tuned to the direction of the correction. Retrieval of a motor program from memory was controlled by a preselection process that turned PCs off and on in a bistable manner. The motor program was then initiated by a trigger signal and continued until the summed proprioceptive and target inputs caused PC transitions to their on-states, whereupon strong inhibition terminated the program. The movement trajectories were qualitatively similar to those observed experimentally. (Supported by ONR N00014-88-K-0339.)

503.14

Computer Animation of Electrophysiological Responses. SA Reid, RA Palovcik, JC Principe and A Albuquerque Jr. Univ. of Florida, Depts. of Neurosurgery and Electrical Engineering and V A Medical Center. Gainesville, FL.

New methods for displaying data from multiple channel recordings plot a dependent variable on a third axis over spatial extent of tissue. The resulting computer-generated surfaces are animated over time to reveal simultaneous patterns of correlated activity over the entire slice.

This method was implemented for displaying evoked potential data recorded from multiple sites in rat hippocampus and human cortex. The animation revealed important dynamic differences between the propagation of evoked activity in hippocampal vs. cortical slices despite the fact that many individual traces from each appeared similar. Our animations instantly communicate complex spatial patterns in data. This allows intuitive comprehension of the dynamics of brain slice neuronal circuitry at a level impossible to achieve with traditional voltage-time plots. The results of our animation provide a new conceptual framework for analyzing multisite evoked and spontaneous activity.

503.15

TEMPORAL PATTERNS IN A CAM. H. Stowell. ERBP Lab., 120 Nature Creek, Milledgeville GA 31061 USA.

How does a Content Addressable Memory (CAM) store and recall the timing of sensory stimuli? Continuing EEG data from humans [1] imply that quasi-limit-cyclic activity in the 'theta-alpha' range (5-15 Hz) is critical to working memory of timing. Five naive subjects, trained to minimize extracranial artifacts, show phase constraints and phase ordering at above-chance level in scalp-conducted, vertex-to-earlobes EEG when attending to novel sequences of auditory transients; this phase ordering appears in both stimulated and silent trials, for small averages and single epochs ($1 \leq N \leq 10$) when subjects have a knowable and self-paced time-frame, but only at chance level within an unknowable time-frame. Momentary drowsing, with failure to recall a wakefully suprathreshold and always detectable acoustic pattern, shows abrupt EEG center-frequency slowing from ~ 10 to ~ 5 Hz before the stimulus time-window and a much larger and later 'vertex evoked potential'. The wakeful effects tend to disappear with subjective familiarity over weekly sessions of 1½-2½ hrs.

1. Stowell, H. Int. J. Neurosci. 32:861; 34:117; 35:111; Int. J. Psychophysiol. 4:219 (1987a-d).

FOR ORIGINAL REPORTS on a presumed time function for human 'vertex EP' see: Clynes, M. in E. Donchin & D.B. Lindsley (Eds) Averaged Evoked Potentials. NASA-Press, DC, 363-374 (1969).

503.16

A MICROELECTRONIC DEVICE FOR MULTICHANNEL STIMULATION OF AND RECORDING FROM NEURONS. E.G. Bylander*[1], R.D. Entreken*[1], H. Moradi*[2], W.M. Gosney*[2], and D.J. Woodward[3]. Bylander Associates[1], Southern Methodist Univ.[2], UT-Southwestern Med. Ctr.[3], Dallas, TX 75235.

An IC chip has been designed and fabricated by MOSIS for alternatively recording from or stimulating each of 16 electrodes placed in the CNS. The chip is based on previous work "VLSI-Circuit Control of Recording and Stimulation in Cultured Neuronal Network Study" by P.V. Vithalani, N.A. Buzaid, R. Balasekaran, W.M. Gosney, L.L. Howard and G.W. Gross (submitted IEEE Transections on Neural Networks). A write-only memory register is included on the chip to select one or more of 16 sensor wires for stimulation, or all inputs can be employed as high impedance sensors. As presently configured, the chip is mounted in a 1 inch square by 1 mm thick leadless chip carrier (LCC) which in turn is wired to a small LCC socket. It is then wired to five 12-pin, 2 mm thick Microtech connectors which allow the unit to be mounted directly to a female Microtech headstage. A testing program has demonstrated successful recording from typical neurons detected by multiple 25 micron microwires implanted in neostriatum of awake behaving rats. The stimulation circuitry also has been successfully tested. The practical dynamic range of biphasic stimuli is +1.2V to -3.8V but may be extended. These results are in use for the design and development of the next generation chip which will include 32 I/O channels, but with reduced I/O lines by means of multiplexing and serial control. Target installations will include embedding of the chip in configurations employing multichannel silicon probes with microcable interconnections. The latter are under development with Dr. S. Ang at U. Arkansas. *Supported by the Communities Foundation of Dallas, MH44337, AFOSR 90-0146, DA02338, and the Biological Humanics Foundation.*

503.17

DYNAMIC CONVERGENCE IN NEURAL ASSEMBLIES. P.H. Bedenbaugh[1], G.L. Gerstein[1], A.M.H.J. Aertsen[2]*, [1] *Departments of Physiology and Bioengineering, University of Pennsylvania, Philadelphia,* [2] *MPI for Biological Cybernetics, Tübingen, FRG*

When interpreting the simultaneously recorded activity of several neurons one must remember that the neurons are embedded in a large, densely interconnected network. The firing of the observed neurons is partly determined by the activity of the many unobserved neurons. For any two observed neurons, the presynaptic network can be subdivided into a shared pool that projects to both cells and pools that project only to one cell. Let us call the influence that a presynaptic pool, shared or unshared, exerts on an observed target neuron the 'dynamic convergence'. In contrast to the static, anatomical notion of a converging projection, dynamic convergence is a physiologic measure that reflects rapid shifts in the active and inactive parts of neural structures. Recent theoretical work has shown that the properties of this dynamic convergence, both its magnitude and its internal correlation structure, dramatically effect the strength of correlation between the activities of the observed postsynaptic neurons.

It is infeasible to directly measure the activities and relative influences of the various presynaptic pools. We can learn something about the unobserved presynaptic pools, and hence the dynamic convergence by studying the joint statistics of the observed spike trains. We are investigating a dynamic generalization of the coherence function. The coherence function is the the cross spectrum of two processes, normalized by the product of the square roots of their individual autospectra. Coherence is widely used in geophysics and EEG-analysis to discern the extent to which two spatially distinct observations are driven by the same underlying process. For linear systems, coherence varies between zero and one and provides a static measure of shared input.

This type of quantitative measure is relevant for evaluating contrasting ideas of neuronal assembly organization. Each neuron in a Hebbian assembly participates in several loosely coupled networks. Input activity imposes a particular organization by igniting one such network. Other models are built on tightly coupled and essentially disjoint groups of neurons whose organization would appear invariant with stimulus conditions. Dynamic convergence would be highly stimulus related in the first case, and not so in the second.

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INVERTEBRATE MOTOR FUNCTION

504.1

THE ROLE OF THE BRAIN IN LOCOMOTORY BEHAVIOR OF A PARASITIC FLATWORM. M.V.K. SUKHDEO. Dept. of Animal Sciences, Rutgers University, New Brunswick, NJ 08903.

The flatworms (phylum Platyhelminthes) are the first phylogenetic group to evolve "true brains" and thus, are appropriate models for the study of the original functions of the brain. *Hymenolepis diminuta*, a parasitic flatworm, is capable of coordinated locomotory activity that is not affected by the removal of its brain. This parasite uses a complex adaptive behavior, consisting of retrograde peristaltic-like waveforms (PL), to maintain its position in the small intestine. In addition, it also uses this behavior to migrate anteriorly in the intestine (in response to cues elaborated during host feeding) to position its strobila in the regions of highest nutrient concentrations. PL frequency varies along the strobila in a coordinated manner, occurring at a rate of 25.3 ± 1.1 Hz in the cephalic region and decreasing linearly to a rate of 5.8 ± 1.3 Hz in the caudal regions. The form and frequency of this complex behavior along the strobila is not significantly altered by decerebration. In addition, when placed on a thermal gradient (20-32°C), intact worms use PL waves to migrate uphill to a preferred temperature of ~30°C (thermo-orthokinesis). Decerebrate worms also move similarly up the gradient, but there are some qualitative differences in the behavior. Although worm survival in the host requires the brain, it is concluded that the elaboration of PL waves and the coordination of these movements along the body is under the control of the peripheral system.

504.2

TEMPERATURE DEPENDENCY OF WING-BEAT FREQUENCY IN INTACT AND DEAFFERENTED LOCUSTS. J.A. Foster and R.M. Robertson. Department of Biology, Queen's University, Kingston, Ontario, K7L 3N6, Canada.

Locusts do not regulate thoracic temperature during flight. As a result the thoracic temperature of a flying locust can exceed ambient by as much as 8-10°C. Elevated thoracic temperatures have been shown to affect wing-beat frequency in intact *Locusta migratoria*. This study investigated the temperature dependency of wing-beat frequency in intact and deafferented *Locusta*.

Intact tethered locusts were flown in a wind tunnel at different ambient temperatures for up to 3 hours while wing-beat frequency and thoracic temperature were monitored. EMG recordings were made in a deafferented locust perfused with different temperature salines. Wing-beat frequency was shown to vary with thoracic temperature in both the intact and the deafferented situation. The slope of the rise in wing-beat frequency with experimental increases in thoracic temperature was similar in intact and deafferented animals.

Thus these experiments demonstrate that a central effect of temperature on wing-beat frequency exists. Further intracellular experiments are necessary to investigate the neural basis of these temperature effects.

504.3

EFFECTS OF TEMPERATURE ON PROPERTIES AND INTERACTIONS OF FLIGHT NEURONS IN THE LOCUST. R.M. Robertson and J.A. Foster. Department of Biology, Queen's University, Kingston, Ontario, K7L 3N6, Canada.

An increase in the thoracic temperature of intact Locusta migratoria tethered flight results in an increase in wingbeat frequency. Similarly, increasing the temperature of the thoracic ganglia in a deafferented preparation results in an increase in the frequency of the centrally generated flight rhythm.

We investigated the neural basis for these changes using a deafferented preparation of Locusta migratoria. Intracellular recordings were made from the neuropile segments of identified flight neurons using standard techniques. The temperature of the ganglia was altered by superfusing them with warmed saline (25-40°C).

To date we have found the following effects which are consistent with previous studies of temperature effects in insect ganglia. The duration and amplitude of action potentials were decreased. The latency of direct synaptic connections was approximately halved by a 10°C rise in temperature. In contrast with previous reports of an increase in the amplitude of excitatory post synaptic potentials (psps) we found that the amplitude of inhibitory psps was decreased. Preliminary evidence indicates that this reduction may be due to membrane hyperpolarization brought on by increased temperature.

504.4

PLASTICITY IN THE FLIGHT SYSTEM OF THE LOCUST. A.Büschges and K.G.Pearson, Dept. of Physiology, University of Alberta, Edmonton, Canada.

The initial depolarization in elevator motoneurons in intact flying locusts is induced by the phasic activity of the hindwing tegulae during the downstroke of the wings. Previous studies have shown that removal of the tegulae results in a decrease in the rate of depolarization in elevators, an increase in the interval between the onset of depressor and elevator activity (the D-E interval), and a reduction in the wing beat frequency. We have now quantified these effects and found that they are not permanent. The average wing beat frequency (WBF) of the intact flying locusts was about 20 Hz and the average D-E intervals were about 20 ms for the forewing and 25 ms for the hindwing. Removal of the wing tegulae induced a drop in WBF to 73% of the initial value and caused an increase of the D-E intervals (average increase D-E interval forewing: 12 ms; average increase D-E interval hindwing: 17 ms). The initial fast and rapid depolarization in the elevator motoneurons disappeared. Over a period of about 2 weeks following the removal to the tegulae there was a progressive increase in the WBF (to about 90% of its intact value) and a progressive reduction in the D-E intervals. 14 days after the removal of the tegulae the &average D-E interval for the forewing was about 20 ms and the average D-E interval for the hindwing was about 28 ms. Intracellular recordings from the elevator motoneurons in recovered animals during flight showed that the initial depolarization was comparable to that occurring in normal intact animals. The neural mechanisms underlying the recovery of the flight pattern following tegula removal are now being investigated. Preliminary data have indicated that there are changes in the characteristics of the central rhythm generating network as well as alterations in the influence from other wing proprioceptors in establishing the motor pattern.

504.5

NEURONES WITH BILATERAL AXONS IN THE LOCUST CENTRAL NERVOUS SYSTEM. H.-J.Pflüger[1], P.Stevenson[1], M.Ferber[1] and M.Eckert[2]. Freie Universität Berlin, Institut für Neurobiologie, Königin-Luise Str. 28-30,D-1000 Berlin 33,FRG[1]) and Univ. Jena,WB Tierphysiologie, Erbertstr.1, DDR-6900 Jena, GDR[2]).

In the thoracic and abdominal ganglia of the locust central nervous system several populations of midline cells with bilateral axons exist, that release neuromodulatory substances. In the abdominal ganglia some of the cells could be identified with respect to their target organs and transmitters. Among the cells with bilaterally symmetrical axons are two "classical" DUM-(dorsal unpaired median) cells that innervate all skeletal muscles of the abdomen and stain with an Octopamine antibody (M.Eckert,Jena). Two other cells with bilateral symmetrical axons, also resembling DUM-cells, run exclusively to the insect heart. One of the two cells stains with an FMRFamide-like antibody. Neurones with bilaterally asymmetrical axons exclusively run to the heart and do not stain with either an Octopamine or FMRFamide-like antibody.

504.6

MEMBRANE PROPERTIES OF LOCUST NONSPIKING LOCAL INTERNEURONES. G. Laurent. California Institute of Technology, Biology Division, 156-29, Pasadena CA 91125.

The electrotonic and active membrane properties of nonspiking local interneurones were studied, using the switched current- and voltage-clamp techniques in neuropilar recordings. The average transmembrane potential (V_r) of the interneurones was 58±6mV (n=85) and the input resistance (in the linear region of the I-V curve) was 16.5±8MΩ (n=19, range 8 to 32MΩ). The charging curves evoked by low density hyperpolarizing current pulse injection yielded 2 time constants (τ_m and τ_l) whose average values were 33.2±9ms and 3.3±1ms (n=18) respectively. The mean specific membrane resistance of the nonspiking interneurones is thus about 33kΩ.cm^2. An outward rectification was consistently observed upon depolarization. This rectification was generally activated from potentials more negative than V_r, and was accompanied by a decrease in input resistance and membrane time constant. The resting membrane, for example had a time constant of 26.4±8ms (n=31). This outward rectification was due to at least 2 conductances with different inactivation kinetics, similar to the A- and delayed rectifier conductances. No inward rectification was observed upon injection of hyperpolarising current. In about 60% of the recordings, an active and TTX-resistant depolarizing process could be evoked by rapid depolarization around V_r. The voltage-dependent properties of the membrane of the nonspiking interneurones had dramatic effects on the time course of natural or evoked unitary PSPs. The half width of EPSPs, for example, decreased by a factor of 7.5 if the membrane potential was shifted from -90 to -50mV. *Supported by The Royal Society, SERC (UK), and the Hasselblad Foundation.*

504.7

DISTRIBUTION OF SYNAPTIC INPUT OF WING STRETCH RECEPTORS IN DENDRITES OF LOCUST FLIGHT MOTONEURONS. H. Schneider, Universitaet Konstanz, Biologie, 7750 Konstanz, FRG.

To study the integrative properties of identified motoneurons (MNs) synaptic input was compared in various cellular stuctures using double intracellular recordings. Overlap of the arborizations of the pre- and postsynaptic neurons was demonstrated by differential intracellular staining (DIS) with Lucifer Yellow and Texas Red.

The morphology of the 2 subalar MNs (SAMNs, wing depressor) in the mesothorax of the locust Locusta migratoria is characterized by 6 primary dendrites (PD) which originate laterally and medially from a neuropilar segment (NPS). In both SAMNs, EPSPs are directly elicited by activity of forewing (msSR) and hindwing stretch receptors (mtSR). These EPSPs were recorded with no latency shift and with similar time course in the PDs and the NPS. Amplitude and time to peak indicate that msSR-EPSPs originate in all recorded PDs; mtSR-EPSPs, however, appear to originate mainly in the medial PDs and spread electrotonically into the lateral PDs. DIS of SRs and SAMNs support these interpretations. The msSR overlap with all recorded PDs; the mtSR, however, mainly with the medial PDs.

These results suggest that a functional differentiation of PDs of SAMNs with regard to synaptic input from SRs does not exist due to the anatomical organization of direct physiological connections and the electrotonic spread of EPSPs. Supported by DFG (Ku 240/14-1,2).

504.8

IN FLIES, MOTOR NEURONS SUPPLYING INDIRECT (POWER) AND DIRECT (STEERING) FLIGHT MUSCLES OCCUPY DISCRETE NEUROPILS SUPPLIED BY CHARACTERISTIC MOTION-SENSITIVE DESCENDING NEURONS. Nicholas J. Strausfeld and Carol Arakaki*. Arizona Research Labs, Division of Neurobiology, Univ. of Arizona, Tucson AZ 85721

Specific flight muscles [nomenclature: Wisser A, Nachtigall W (1983) Zoomorphology 104:188-195] have been impaled with cobalt-filled electrodes to retrogradely fill motor neurons supplying them. Silver-intensified neurons were reconstructed, and related to specific depths and regions within thoracic ganglia. Two sets of fibrillar muscles (DLMs and DVMs) and one set of non-fibrillar muscles [hg1, 2, pro- and supinators. Functions: Wisser and Nachtigall (1983); Heide G (1968) Z Vergl Physiol 59:456-460] are supplied by superficial pterothoracic motor neurons whose bilaterallay symmetrical dendrites are visited by bilateral terminals of descending neurons (DNs) responding to symmetrical front-to-back visual flowfield motion [see Gronenberg and Strausfeld (1990) Neurosci Abstr 16]. At deeper levels in the meso- or metathoracic ganglia, large unilateral, or bilaterally asymmetric motor neurons supply antagonistic basalar muscles involved in steering. These neurons are visited by terminals of DNs that respond to asymmetrical shifts of the visual panorama, or to small-field motion. Laterally and ventrally, unilateral motor neurons [examples: I_1, III_2, Tp) include those involved in tension and wing-vault control. Certain steering motor neurons (B1-3, III_1) show cobalt-coupling to specific functionally identified DNs and to primary sensory afferents from the wings and halteres, thus providing evidence for the convergent role of synergistic mechanosensory and visual inputs in flight maneuvers. **Supported by NIH Grant No. R01 EYO 7151-01 and NSF Biological Centers Grant DIR 82-20082**

504.9

IN FLIES, FLIGHT VELOCITY AND STEERING MANOEUVRES RELATE TO TWO DIFFERENT SETS OF DESCENDING NEURONS.
Wulfila Gronenberg and Nicholas J. Strausfeld. Arizona Research Labs, Division of Neurobiology, Univ. of Arizona, Tucson AZ 85721

Neuroanatomical studies of Diptera show that descending neurons (DNs) originating from dorsal brain neuropil connect to the flight motor neuropil in the thoracic ganglia. A variety of staining techniques reveal that these dorsal DN are postsynaptic to 1) "giant" motion sensitive lobula plate tangential (VS and HS cells); 2) "small" lobula plate tangentials; 3) retinotopic neurons; 4) mechanosensory afferents. The DN dendrites are organized in 4 groups (dorsal DN clusters) each of which receives input from a different set of lobula plate interneurons. The terminals of certain dorsal DNs arborize bilaterally in the dorsalmost meso- and metathoracic neuropil which contains bilateral motor neurons of the indirect flight power muscles providing thrust. Most dorsal DNs terminate unilaterally in dorsolateral neuropil of all three thoracic neuromeres. These contain motor neuron dendrites of the neck muscles and of the direct flight steering muscles. Intracellular recordings reveal that DNs with bilateral terminals respond best to symmetric visual flowfields such as encountered during straight flight. DNs that terminate in the neck- and flight steering motor neuropil prefer directional stimuli such as downward or horizontal motion or non-symmetric visual flowfields. The same stimuli trigger muscle activity in steering muscles, as recorded electromyographically, and induce behavioral optomotor responses (pitch, yaw, and roll). We conclude that DNs extract specific features from the common pool of input information and process this information with respect to its relevance to the control of a particular flight manoeuvre. **Supported by NIH Grant No. R01 EYO 7151-01**

504.10

PROJECTION NEURONS FROM THE THIRD OPTIC NEUROPIL OF FLIES SEGREGATE ONTO DISCRETE CLUSTERS OF RELAY NEURONS DESCENDING TO THE THORACIC MOTOR NEUROPIL
Cole Gilbert and Nicholas J. Strausfeld. Center for Insect Science and ARL-Div. Neurobiology, Univ. Arizona, Tucson, AZ, 85721.

In Diptera the third optic neuropil is divided into a ventral lobula and a dorsal lobula plate. Neuroanatomical studies using Golgi impregnation or cobalt backfilling show that uniquely identifiable neurons or assemblies of neurons from the two regions project to the lateral deutocerebrum to separate target areas which are associated with discrete clusters of descending neurons (DNs). These integrate afferent visual, as well as olfactory and mechanosensory, information and project to thoracic motor neuropil. Intracellular recording and lucifer yellow dye injection has been used to characterize physiologically and identify anatomically visual neurons projecting to DN clusters. The dorsal clusters receive input from small- and wide-field neurons arising in the ipsilateral lobula plate. Most, but not all, afferents are maximally excited by specific directions of moving stimuli. The ventral clusters receive input from wide- and small-field neurons arising in the ipsilateral lobula, and in males but not females, from a sex-specific neuron projecting from the contralateral lobula. These afferents are also of several physiological classes, with some being directionally-selective, but many being more sensitive to light ON and OFF than to motion. The physiologically determined receptive fields of afferents from the lobula or lobula plate are approximately congruent with the dendritic spreads in the retinotopic projection of visual space into the neuropil. The differences in response properties of afferents apparently relate to the destination of specific DNs to their thoracic target areas. **Supported by a Center for Insect Science Postdoctoral Traineeship to CG (NSF DIR 82-20082) and an NIH Grant R01 EY07151 to NJS**

504.11

MUTATIONS AFFECTING K AND Na CURRENTS IN *DROSOPHILA* ALTER PHYSIOLOGICAL PROPERTIES AND PRODUCE ABNORMAL SPONTANEOUS ACTIVITY IN THE GIANT FIBER PATHWAY.
J. E. Engel and C.-F. Wu. Dept. of Biology, University of Iowa, Iowa City, IA 52242.

The neural circuitry controlling the escape reflex in flies has been well characterized. We examined the physiological and behavioral effects of perturbing this pathway with the mutations *Shaker* (*Sh*), *Hyperkinetic* (*Hk*), and *ether-á-go-go* (*eag*), which reduce K currents, and *no action potential* (*nap*), which reduces Na current. By recording from the dorsal longitudinal (DLM) flight muscles and the tergotrochanteral (TTM) leg extensor muscle, we monitored spike activity underlying a jump-and-flight escape response to electric stimulation mediated by the giant fiber pathway.

The mutants *Sh*, *Hk*, *eag Sh*, and *Hk eag*, which affect K currents, have a longer refractory period for both DLM and TTM response, with latency unchanged. In contrast, *nap* flies show lengthened latency and refractory period, but only in the TTM . Apparently the TTM pathway is preferentially affected because of differential expression of the *nap* gene or a higher sensitivity due to a lower safety margin in a component of the pathway.

Double mutant *eag Sh* flies show abnormal spontaneous rhythmic spiking in DLM muscles. This is not associated with wing beating, nor does it respond to tactile stimuli that turn flight on and off. The non-flight spikes are not seen in *eag* or *Sh* alone and are suppressed by *nap* in *eag Sh; nap* triple mutants. Simultaneous recordings from pairs of DLM muscle fibers innervated by the same or separate giant fibers indicate that the spontaneous activity is neurogenic and may involve the flight pattern generator or an interneuron mediating the giant fiber response. In *Hk eag*, rhythmic spiking is seen in the TTM in addition to the DLM, implicating the giant fiber , which innervates both muscles.

504.12

CHANGES IN PROPRIOCEPTIVE CIRCUITS DURING INSECT METAMORPHOSIS.
D.A.Tamarkin and R.B.Levine. ARL Div. Neurobiology, Univ. Arizona, Tucson, AZ 85721.

During insect metamorphosis there are dramatic changes in behavior that require alterations in neural circuits. Toward understanding this reorganization, we have characterized the interactions between the bilaterally-paired abdominal stretch receptors (SRs) and identified motoneurons (MNs) in the larval and adult stages of Manduca. The SR was left attached to the isolated nervous system, and stimulated electrically. MNs were monitored intracellularly and identified by lucifer-yellow injection. Our initial studies have focused on the intersegmental muscle (ISM) MNs which persist through adult emergence and show relatively little dendritic alteration.

In both the larva and pharate adult, ISM MNs with **bilateral** dendrites receive excitatory input from ipsi. and contra. SRs in the same segment as the target muscle. Signal averaging and treatment with high-divalent saline suggest that these connections have a direct component. ISM MNs with **unilateral** dendrites receive direct excitatory input from only the ipsi. SRs in both stages. The input to these unilateral MNs from the contra. SR is polysynaptic in both stages, but is inhibitory in the larva and changes to excitatory during adult development. Similar, but weaker and more variable, connections were observed for MNs innervating the next posterior segment. All of these connections are consistent with opportunities for the SR terminals and the MN dendrites to overlap at the light level. Interneurons which receive direct input from the SR have been identified. These may be involved in the polysynaptic connections described.

Thus, significant changes in the proprioceptive information supplied to the ISM MNs occur during metamorphosis. These changes may be related to the changes in abdominal posture and movements seen during this period.

504.13

MODELLING THE CONTROL OF INSECT SKELETAL MUSCLE.
Jim H. Belanger. Dept. of Zoology, Univ. of Toronto, Toronto, Canada M5S 1A1.

Vertebrates and invertebrates use fundamentally different strategies for muscular control. Vertebrates use many motor units, with very little modulation of individual units, while invertebrates use very few units (often only one or two) combined with extensive peripheral modulation. This renders much of the existing modelling work on vertebrate muscle inappropriate for application to insects. I have attempted to develop a relatively low-level model which takes into account the extensive modulatory input to insect skeletal muscle.

The model is a numerical simulation written in C and run on an IRIS workstation. The essence of the model is the production of graded contraction via the progressive recruitment of radially-arrayed Ca^{++} release sites. Potential changes at the surface of the model fibre passively propagate inward along the invaginated tubules, triggering localized release of Ca^{++} whenever the excitation-contraction threshold is reached. Several sites within the model are suitable loci for modulation. The most powerful effect is produced by lowering the excitation-contraction coupling threshold, which can produce contraction in the absence of depolarization (as is seen, for instance in the action of the peptide cotransmitter proctolin in many systems (Orchard, Belanger and Lange, *J. Neurobiol. 20*, 1989)). Other available modulatory mechanisms are ligand-gated calcium channels in the sarcoplasmic reticulum (SR), which are in parallel with the voltage-gated channels, and the Ca^{++}-uptake system of the SR.

By varying the nature of the synaptic input, this model allows an examination of the nonlinear interactions between the conventional (excitatory and inhibitory) transmitters and the unconventional cotransmitters. In addition, it allows quantitative examination of the hypothesis that cotransmitters are used for energy efficiency in motor control.

504.14

GABAERGIC PRESYNAPTIC INHIBITION AS MODULATOR OF MONOSYNAPTIC REFLEX IN CRUSTACEA.
A. EL MANIRA*, D. CATTAERT* & F. CLARAC. CNRS LNF2, 31 Chemin Joseph Aiguier BP 71, 13402 MARSEILLE CEDEX 9 FRANCE.

In the thoracic crayfish *in vitro* preparation, it is possible to record intracellularly sensory terminals from a complex leg proprioceptor (the chordotonal CB) and motoneurons (MNs) controlling the joint where this receptor is inserted. Mechanical as electrical stimulation applied on the CB receptor induces monosynaptic resistance reflex in MNs. By recording from CB terminals, we have demonstrated the presence of primary afferent depolarizations (PADs). In tonic preparation PADs occur continuously, by contrast during fictive locomotion they occur at fixed time in the locomotor cycle. Spontaneous PADs were supressed by bath application of GABA antagonists (Picrotoxin and Bicuculline). This result suggests that GABA receptors exist in the presynaptic CB terminals. This hypothesis was verified since small quantities of GABA or Muscimol applied near the recording site over the ganglion elicit a transient depolarisation of the impaled sensory terminal; they were coincident with a reduction of input resistance. The inhibitory role of GABA on the monosynaptic reflex has been confirmed since all MNs showed a marked and reversible reduction in compound EPSP amplitude when the CB nerve was stimulated during GABA application.

504.15

COMPONENTS OF REFLEX STIFFNESS IN HERMIT CRAB ABDOMINAL MUSCLE. W. D. Chapple. Dept. of Physiology and Neurobiology, University of Connecticut, Storrs, CT 06269.

Control of shell position in the hermit crab is mediated in part by a stretch reflex in the abdominal ventral superficial muscles (VSM). By stretching the muscle with band-pass limited (25 Hz) Gaussian noise and sampling muscle force and membrane potential, the input-output properties of the reflex can be determined by parametric identification techniques. After a transient increase in stiffness and 4th segment motoneuron frequency at stimulus onset, mechanical impedance decays to a steady-state level after 10 seconds and can be approximated by a third order model. Cutting the abdominal connectives at different segments produce changes in the impedance that suggest that there is a low frequency component (0.1 Hz) possibly due to interneuron activation and a higher frequency component (1.0 Hz) that may be due to a direct sensory pathway. The changes at low frequencies are consistent with an involvement of both excitatory and inhibitory pathways in controlling impedance.

504.16

SEROTONIN (5-HT) POTENTIATES CONTRACTION OF DISSOCIATED FIBERS OF APLYSIA BUCCAL MUSCLE. F. Zhang and J.L. Ram, Dept. of Physiology, Wayne State Univ., Detroit, MI 48201

Buccal muscles of *Aplysia* are innervated by cholinergic and serotonergic neurons. Serotonergic input underlies, in part, increased contractility during feeding arousal. With isolated muscles, acetylcholine (ACh) causes contraction, whereas 5-HT potentiates contraction. To study cellular mechanisms underlying these responses, muscle fibers were dissociated with collagenase (2-4 hr at 30 °C). Fibers average 270 ± 10 μm in length (n=37) and 10.8 ± .7 μm in diameter (n=19; max. diameter averages 13.6 ± .9 μm). High K (100 mM superfusion for 2 s) causes contraction, e.g. shortening a typical field of 10 fibers from 311 ± 20 μm to 262 ± 17 μm ($p<.05$, paired t). 1 μM nifedipine blocks High K contraction. 5-HT (10^{-6} M, 1 min) potentiates contraction (resting length, 285 ± 35 μm; 3 s high K, 190 ± 30 μm; 3 s high K after 5-HT, 140 ± 20 μm; n=5, $p<.05$, paired t). ACh also elicits contractions which are potentiated by 5-HT: resting length, 264 ± 20 μm; 3 s ACh, 194 ± 15 μm; 3 s ACh after 5-HT, 156 ± 13 μm; n=6, $p<.05$, paired t. Contraction is also elicited by depolarization via patch electrode. Whole cell currents elicited by depolarization and agonists are described in another abstract. Experiments are in progress to determine whether contractions elicited by patch electrode can be potentiated by 5-HT. Supported by MDA and NIH RR 08167.

504.17

INDIVIDUAL BUCCAL MOTONEURONS OF *APLYSIA CALIFORNICA* INNERVATE MULTIPLE BUCCAL MUSCLES AND DISPLAY UNIQUE NEUROMUSCULAR SYNAPTIC PLASTICITIES. K. P. Cohen* and M. D. Kirk. Div. Biol. Sci., Univ. Missouri-Columbia, Columbia, MO 65211.

Several buccal motoneurons of *Aplysia californica* and their target muscles of the buccal mass have been previously identified. For instance, B15 and B16 were shown (Cohen et al., 1978) to innervate the Accessory Radular Closer (ARC) Muscle also known as muscle I5 (Howells, 1942). However, using a semi-intact preparation consisting of the buccal ganglia attached to the buccal mass (muscles I1-I6 exposed) via nerves n4 and n5, we have shown that several of these large ventral cluster motoneurons innervate more than one nonhomologous buccal muscle. In addition to innervating I5, B15 innervates I4 and B16 innervates I1/I3, I4 and I6. Furthermore, B6 and B9 innervate I1/I3, I4 and I6, B11 innervates I1/I3, I4, I5 and I6 while B10 innervates only the bilaterally homologous I1/I3 muscles. At 10 Hz stimulation to individual motoneurons, neuromuscular synaptic potentials exhibited facilitation and depression with amplitudes and time courses unique to particular motoneurons. From these results it is clear that to understand the behavioral significance of firing in particular motoneurons, the co-activation of nonhomologous as well as bilaterally homologous buccal muscles by individual motoneurons must be considered. Supported by NIH grant NS24662.

504.18

IDENTIFICATION AND CHARACTERIZATION OF BUCCAL MOTONEURONS EXPRESSING FMRFAMIDE-LIKE IMMUNOREACTIVITY IN *APLYSIA CALIFORNICA*. T. L. Ross and M. D. Kirk. Div. Biol. Sci., Univ. Missouri-Columbia, Columbia, MO 65211.

In *Aplysia californica* the peptide FMRFa is synthetized by several buccal neurons (Lloyd et al., 1987) and has physiological actions in the feeding system. However, the identities and targets of neurons synthesizing FMRFa are unknown. We are using intracellular lucifer yellow injections combined with immunocytochemistry to uniquely identify buccal motoneurons expressing FMRFa-like immunoreactivity (FMRFa-LI).

Large ventral cluster cells of the buccal ganglia from 150 to 300 gram animals were identified based on cell body size and position, axonal projections, and muscle innervation. Following ionophoretic injection of these cells with lucifer yellow, the ganglia were prepared as either 10 micron paraffin sections or whole mounts. Preliminary double-label experiments show that the motoneuron B3 expresses FMRFa-LI and projects to the ipsilateral buccal muscle I1/I3. Future experiments will identify the remaining motoneurons expressing FMRFa-LI and test the properties of their neuromuscular synapses. Supported by NIH grant NS24662.

504.19

NEURONAL CONTROL OF COORDINATED LIP MOVEMENTS DURING FEEDING IN *APLYSIA*. S. C. Rosen, E. L. Halvorsen*, E. C. Cropper, M. W. Miller, K. R. Weiss, and I. Kupfermann. Cntr. Neurobiol. & Behav., Columbia Univ. & NYS Psychiatric Inst., New York , NY 10032.

Ingestive behaviors involve coordinated head, lip, and buccal mass movements. Buccal movements are controlled by motor neurons and a central pattern generator (CPG) in the buccal ganglion. In order to study the integration of lip and head movements, we identified lip motor neurons (C11 and C12) that have somata in the cerebral ganglion M clusters and axons in the ipsilateral lower lip (LLAB) nerves. The cells receive direct synaptic inputs from C2 and C3, histaminergic mechanoafferents with receptive fields adjacent to the lips, and B19, a buccal to cerebral interneuron (BCI). C12, but not C11, showed remarkably diverse immunocytochemical staining for neuropeptides found in various buccal motor neurons including, SCPb, buccalin, and myomodulin. The firing of C11 produced circumferential contraction of the inner lips. Firing C12 produced radial contraction that extended to the neck. LLAB lesions produced deficits in inner lip movements during feeding by free-moving animals, whereas upper lip nerve lesions did not. Chronic nerve recordings showed that C11 fired during lip closure, prior to radula protraction, and C12 fired during lip opening and radula retraction. When feeding programs were driven by stimulating cerebral command element CBI-2, C11 and C12 fired antiphasically at rates sufficient to elicit feeding movements. The buccal programs incorporated activity of cerebral motor neurons for extrinsic buccal muscles, but had no effect on cerebral B cluster lip motor neurons. When synaptic transmission in the buccal ganglion was blocked by divalent cations, or when the cerebral-buccal connectives were cut, CBI-2 no longer phasically activated C11 or C12, although it produced weak tonic activation of C11. The findings suggest that coordinated lip movements for feeding are produced by cerebral motor neurons that are driven by a buccal CPG network via BCIs. Moreover, expression of the motor program may be modulated by local cerebral circuitry.

504.20

COMPARISON OF THE BEHAVIORAL EFFECTS OF ENDOGENOUS PEPTIDES ON VISCERAL MOTOR CIRCUITS IN APLYSIA. JLM Morgan, SH Ligman, PH Brownell. Dept of Zoology, Oregon St Univ, Corvallis, OR 97331

In *Aplysia*, the behavioral functions of neuropeptides can be studied in the context of simple motor circuits that control discrete components of visceral motor behavior. Of particular importance as a model is the neuroendocrine bag cell system that triggers egg-laying behavior. Peptides released by the bag cells affect many neurons in the abdominal ganglion, but the connection between neuronal and behavioral effects has not been systematically examined. We have used a semi-intact preparation of branchial and cardiovascular organs to investigate this relationship: By 1) evoking bag cell discharges, and 2) infusing the bag cell peptides, egg-laying hormone (ELH) and α-bag cell peptide (α-BCP) into the abdominal ganglion artery, we have quantified this relationship for four motor circuits. These motor circuits vary in complexity from a single motor neuron regulating tonic muscle tension (pericardial membrane) to several neurons (interneurons and motor neurons) regulating complex patterned movements (vascular, gill, and siphon motor systems). In the simplest circuit, α-BCP and ELH caused changes in spike rate of the motor neuron (LC_P) that were closely correlated with motor output (pericardial tension). In the more complicated gill and siphon circuits, the bag cell peptides affected most of the motor neurons involved, but not in a manner that accounted for the observed behavior. For example, α-BCP inhibited nearly all gill and siphon motor neurons, but failed to relax branchial muscle tension. In some preparations vascular infusions of α-BCP reduced the magnitude and or frequency of respiratory pumping. Similarly, vasomotor neurons (LB_{VCa}) were strongly inhibited by α-BCP without relaxation of vasomotor tension. We conclude that the central effects of peptides on motor neurons can be directly correlated with behavior in simple circuits. However, in larger circuits, the responses of a more complicated system are increasingly influenced by interneurons that integrate activities of these cells. (NIH NS18681, OR Heart Assoc. 80-962, Sigma Xi)

504.21

PERIPHERAL MODULATION OF SWIMMING SPEED IN A PTEROPOD MOLLUSC. R. A. Satterlie. Deparment of Zoology, Arizona State University, Tempe, AZ 85287-1501.

Three swimming speeds have been described for the pteropod mollusc Clione limacina: slow, fast and escape. The change from slow to fast swimming can be attributed to changes in the configuration of the pattern generator, and the recruitment of fast-twitch motor units, as opposed to the activation of only slow-twitch motor units during slow swimming. Escape swimming is apparently superimposed on fast swimming since additional changes in pattern generator and swim motor neuron activity have not been noted. Escape swimming appears to involve activity in two sets of neurons that modify the activity of the swim musculature. In both sets of neurons, electrical activity is independent of pattern generator activity. One set of neurons monosynaptically excites both slow- and fast-twitch muscle cells and produces strong wing contractions. The other neurons show serotonin-immunoreactivity and produce a short-term excitatory modulation of muscle contractility. The time courses of activity in these two groups of neurons suggest that the motor neurons may be used for the initiation of escape swimming (startle response) while the modulatory neurons may be used for maintenance of escape swimming.

BASAL GANGLIA AND THALAMUS VII

505.1

HUMAN CAUDATE AND PUTAMEN CHEMOARCHITECTURE N. Selden, C. Geula, and M-M. Mesulam, Harvard U., Boston, MA

Human striatal neurons containing the peptides choline acetyltransferase (ChAT), somatostatin (SOM) and calcium-binding protein (calbindin D_{28k}) were visualized immunohistochemically using specific antibodies (generously provided by L. Hersh, R. Benoit and M. Celio, respectively).

Each peptide was associated with a different neuronal population. ChAT-positive neurons were relatively large (diameter: 25-35u), multipolar in shape, intensely stained and exhibited no major variations in density between various components of the striatum. SOM-positive neurons were also darkly stained, but were smaller (15-20u) and variable in perikaryal morphology. The density of SOM neurons was higher in the caudate nucleus than in the putamen. Two populations of calbindin-positive neurons were observed: (1) a large population of lightly stained, small neurons (10-15u), distributed throughout the striatum, with the greatest density over the dorso-medial caudate nucleus, 2) a small population of larger (10-20u), darkly stained neurons, most frequently encountered in the lateral putamen. Approximately half of the latter neurons also stained positively for NADPH-diaphorase, an enzyme which is co-localized with somatostatin in striatal neurons. There may thus be an overlap between some of the somatostatin-positive and larger calbindin-positive neurons in the putamen.

These chemoarchitectonic differences may be related to well-known differences of connectivity and behavioral affiliation between the caudate nucleus and putamen.

505.2

IMMUNOSTAINING FOR PROTEIN 10 CALCIUM-BINDING PROTEIN FORMS STRIOSOME-RELATED PATCHWORK IN THE RAT STRIATUM. B. Quinn, A.M. Graybiel, L. Winsky, and D.M. Jacobowitz. Dept. of Brain & Cognitive Sciences, Massachusetts Institute of Technology, Cambridge, MA 02139, and Lab. of Clinical Science, NIMH, Bethesda, MD 20892.

Protein 10 (Pr10) is a newly described calcium-binding protein that is distinct from calbindin 28kD and that may be the mammalian homologue of calretinin (Winsky et al., PNAS 86:10139, 1989). We have studied the distribution of Pr10 immunostaining in the striatum of adult rats, and have compared patterns of Pr10 immunoreactivity to those of calbindin 28kD (CaBP)-like immunoreactivity, a known marker for a large fraction of the small-to-medium-sized neurons of the striatal matrix (Gerfen et al., PNAS 82:8780, 1985). Cellular Pr10 immunostaining appeared in scattered striatal neurons. Neuropil immunostaining for Pr10 was concentrated in patches (ca. 100 um wide) with angular profiles scattered through the caudoputamen, and in the caudoputamen's dorsolateral rim. The most darkly stained Pr10-positive patches were in the dorsolateral part of the striatum, where CaBP immunostaining is minimal. Elsewhere, scattered Pr10-immunoreactive patches appeared to correspond to CaBP-poor zones (patches, striosomes). There was no clear relationship between the locations of the Pr10-positive patches and the Pr10-positive neurons. Heterogeneous distributions of Pr10-positive neuropil also appeared in the nucleus accumbens-ventral striatum. These findings indicate that Pr10 calcium binding protein and calbindin 28kD have different and at least partly complementary distributions within the striosomes and matrix of the striatum. We thank the National Parkinson Foundation & NIH NS 25529, and P.C. Emson for anti-CaBP antiserum.

505.3

EFFECTS OF PRENATAL METHYLAZOXYMETHANOL (MAM) ADMINISTRATION ON STRIATAL PATCH FORMATION. A.M. Snyder-Keller. Wadsworth Center for Laboratories and Research, New York State Dept. Health, Albany, NY 12201.

The formation of striatal patches, consisting of clusters of neurons and overlapping patches of nigrostriatal dopamine (DA) afferents, occurs prenatally. Persistence of the cellular patches after the prenatal removal of DA innervation (Snyder-Keller, Neurosci. Abstr. 15:906, 1989), suggests that intrinsic factors guide the association of patch neurons. Striatal patch neurons are born earlier (E13-15 in rat) than neurons of the matrix (E15-birth) (van der Kooy & Fishell, Brain Res. 401:155, 1987), and this fact was used to selectively delete striatal patch neurons. The antimitotic agent MAM, delivered as a single dose (20-30 mg/kg) or two doses (total $\leq$45 mg/kg) on embryonic days 12-14, resulted in a reduction in forebrain mass and distortion of the striatum. Striatal cellular patches, characterized as substance P-dense and calbindin-poor, were still apparent, but consisted of a smaller number of relatively larger patches. These same neuronal patches could be selectively labelled by an injection of Fluoro-Gold into the neonatal striatum. The DA innervation appeared less distinctively patchy during the first postnatal week, but AChE-positve neuropil assumed a fairly normal distribution. Thus, patches form despite the deletion of a large number of striatal patch precursors, but the birthdate of the neurons remaining in patches remains to be determined. (Supported by Tourette Syndrome Association.)

505.4

VITAMIN D-DEPENDENT CALCIUM-BINDING PROTEIN (CALBINDIN-D-28K) IMMUNOREACTIVE NEURONS IN THE MONKEY AND THE RAT BASAL FOREBRAIN. H.T. Chang, H. Kuo and Q. Tian* Department of Anatomy and Neurobiology, The University of Tennessee, Memphis, College of Medicine, Memphis, TN 38163

A recent study has shown that many cholinergic neurons in the human nucleus basalis of Meynert (NBM) are immunoreactive for calbindin-D-28k (CBP), a Vitamin D-dependent calcium binding protein (Ichimiya et al., Brain Res. 1989, 499:402-406). However, the relationship of the CBP immunoreactive neurons with the cholinergic NBM neurons in other species has remained unclear. In this study, a mouse monoclonal antibody raised against CBP (a gift from Dr. M.R. Celio) and a rabbit antiserum raised against human placental choline acetyltransferase (ChAT) (Chemicon Inc.) were used in double-labeling immunofluorescence reactions to compare the distribution of CBP neurons with that of the ChAT neurons. In the Rhesus monkey (Macaca mulatta) NBM, virtually all of the CBP neurons contain ChAT, whereas about 70 to 80% of the ChAT neurons contain CBP. On the other hand, CBP does not co-localize with ChAT in the rat (Sprague-Dawley) NBM. The rat CBP neurons are fewer in number and smaller in size than the ChAT neurons. The difference in the expression of CBP immunoreactivity in the rat and monkey NBM neurons suggests that Vitamin D-dependent calcium homeostasis may have different roles in the primate and the rodent basal forebrain functions.

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505.5

SEROTONERGIC INNERVATION OF THE RAT BASAL FOREBRAIN: A LIGHT AND ELECTRON MICROSCOPIC IMMUNOCYTOCHEMICAL STUDY. H. Kuo and H.T. Chang, Department of Anatomy and Neurobiology, The University of Tennessee, Memphis, College of Medicine, 875 Monroe Ave., Memphis, TN 38163

In the basal forebrain, non-cholinergic elements may play an important role in regulating the normal activity of cholinergic neuron. However, the distribution patterns and the synaptic connections of many non-cholinergic elements are not known. In this study, the distribution of serotonin (5-Hydroxytryptamine, 5HT) immunoreactive (5HT+) axons in the rat basal forebrain was examined. At least two types of 5HT+ axons could be distinguished in this region: One type consisted of relatively thin fibers endowed with small varicosities, and was found densely distributed throughout the substantia innominata (SI), ventral pallidum (VP), and ventral striatum (VS) without apparent differential distribution. The other type consisted of thicker fibers with larger varicosities, and was found mainly within SI and a junctional area between VP and VS. Electron microscopic analysis revealed that 5HT+ boutons formed asymmetrical synapses with mainly dendrites, and occasionally with somata of SI, VP and VS neurons. Present results suggest that the basal forebrain is innervated by at least two types of 5HT+ axons and that these 5HT+ axons may form excitatory synapses with basal forebrain neurons.

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505.6

CALBINDIN-D28K AND PARVALBUMIN IN PRIMATE BASAL GANGLIA. P.-Y. Côté, A.F. Sadikot and A. Parent. Lab. of Neurobiol., Fac. of Med., Laval Univ., Québec, Canada.

The distribution of cell bodies expressing either calbindin-D28k (CB) or parvalbumin (PV) immunoreactivity (IR) in basal ganglia of squirrel monkeys (*Saimiri sciureus*) was studied on contiguous sections incubated with monoclonal antibodies against CB or PV (M.R. Celio). In the striatum, medium-sized CB-IR cells occurred in very large number and appeared strictly confined to the extrastriosomal matrix, as identified by its intense acetylcholinesterase staining on adjacent sections. Less numerous medium-sized PV-IR neurons were also distributed in a patch-like manner in the striatum, but the correspondence with the striosomal organization was not clear. The CB-IR neurons in the dorsal portion of the striatum were less intensely stained than those in the ventral portion, whereas the inverse occurred for neurons expressing PV. At the pallidal level, neurons in both segments were devoid of CB-IR but displayed very strong PV-IR. The neurons of the subthalamic nucleus were also markedly enriched with PV but displayed only very light CB staining. In the substantia nigra (SN)-ventral tegmental area (VTA) complex, CB-IR cells abounded in the VTA and in the dorsal tier of the pars compacta of SN, but were absent in the ventral tier of the pars compacta and in the entire pars reticulata of SN. In contrast, numerous PV-IR neurons occurred in pars reticulata and pars lateralis of SN, but none were found in the pars compacta and VTA. These findings reveal that the patterns of CB and PV distribution in primate basal ganglia are strikingly complementary, suggesting a synergic role for these two calcium binding proteins in basal ganglia function. [Supported by grants from MRC and FRSQ].

505.7

LIMBIC SYSTEM ASSOCIATED MEMBRANE PROTEIN (LAMP) IN PRIMATE BASAL GANGLIA. A.F. Sadikot, A. Parent and P. Levitt. Lab. of Neurobiol., Fac. of Med., Laval Univ., Québec, Canada and Dept. Anat., Med. Coll. Pennsylvania, Philadelphia, PA

A monoclonal antibody raised against LAMP was used to study the distribution of this glycoprotein in basal ganglia of 2 squirrel monkeys (*Saimiri sciureus*) and 1 cynomolgus monkey (*Macaca fascicularis*). In both species a similar heterogeneous distribution of LAMP immunoreactivity (IR) was seen. In striatum, areas of intense IR included the ventral striatum, rostral and ventral portions of putamen and most of caudate nucleus. The striosomes, as identified on adjacent sections stained for acetylcholinesterase or calbindin-28kD, were much more intensely stained than the extrastriosomal matrix. The external pallidum showed dense IR in its rostral pole and in dorsal and ventral thirds of its more posterior portion. The internal pallidum was virtually devoid of IR, except for a dense zone at its interface with the lateral hypothalamus. The ventral pallidum displayed moderate IR surrounding the more lightly stained subcommissural pallidum. In the subthalamic nucleus LAMP-IR was light laterally but increased significantly medially. In the brainstem, moderate LAMP-IR occurred mainly within the substantia nigra (SN), the ventral tegmental area, the retrorubral field and the pedunculopontine area. The SN pars reticulata displayed rostrocaudal and mediolateral gradients of decreasing IR. The SN pars compacta showed moderate IR in its dorsal tier but only light staining in its ventral tier, whereas the SN pars lateralis was largely devoid of IR. Thus, LAMP-IR is intense in limbic areas, light to moderate in associative territories, and virtually absent in sensorimotor regions of primate basal ganglia.

505.8

RECIPROCAL CONNECTION BETWEEN THE TWO PALLIDAL SEGMENTS IN PRIMATES. L-N. Hazrati and A. Parent. Lab. of Neurobiol., Fac. of Med., Laval Univ., Québec, Canada.

Studies with the lectin *Phaseolus vulgaris*-leucoagglutinin (PHA-L) revealed the existence of interconnections between the external (GPe) and internal (GPi) segments of the pallidum in the squirrel monkey (*Saimiri sciureus*). Small iontophoretic injections of PHA-L in the dorsomedial half of GPe produced significant anterograde fiber labeling in GPi. Both coarse and smooth labeled fibers arose from the injection site, traversed the internal medullary lamina and invaded the dorsomedial half of GPi. The coarse fibers passed through GPi to terminate within the subthalamic nucleus and the substantia nigra. In contrast, the smooth fibers consisting of thin and nonvaricose axons terminated directly within GPi. They were poorly branched and arborized in a pericellular, basket-like pattern around the somata and proximal dendrites of GPi neurons. Typically, one perikaryon was innervated by a single axon displaying numerous rather large varicosities reminiscent of terminal boutons. Conversely, PHA-L injection in the central core of GPi led to significant anterograde fiber labeling in the dorsomedial half of GPe. These fibers displayed long intervaricose segments proximally, and branched rather frequently distally making contact *en passant* with several GPe cells. Hence, the GPi-GPe projection appears rather diffuse in comparison to the more direct cell-to-cell relationship displayed by the GPe-GPi projection. Current electron microscopic studies should reveal more about the type of contact established by this short pallidopallidal interconnection system, which could play a crucial role in the functional organization of the basal ganglia. [Supported by grants from the MRC and FRSQ].

505.9

THE EXISTENCE OF A MARGINAL DIVISION IN THE MONKEY STRIATUM. S.Y.Shu and X.Bao. Dept.of Neurobiology,Inst.of Neuroscience,Xian, China.

In the rat, a band of densely packed medium sized fusiform cells has been found at the caudal border of the striatum and named marginal division.(Shu,et al. J Chemical Neuroanatomy,1988). The marginal division has special morphology, immunohistochemistry and projection patterns, distinguishable from the rest of the striatum.

The present study is to investigate whether a marginal division is in the monkey striatum. A part of monkey(Macaca mulatta) brain including putamen and globus pallidus was sectioned and the sections were processed immunohistochemistry of L-enkephalin(L-ENK), Neurotensin(NT), and Cholecystokinin(CCK). L-ENK, CCK and NT immunoreactive fibers and terminals were more densely accumulated in the caudo-medial border of the putamen than in the rest of the putamen. The neuronal somata are mostly fusiform in this region and some of them are L-ENK and NT immunoreactive. Based on the morphology, immunohistochemistry, and position of this region, it is very possible that a special marginal division, similar to the marginal division of the rat,also exists in the caudo-medial border of the putamen adjacent to the globus pallidus in monkeys.

550.10

EM STUDY OF SP+ STRIATAL NEURONS AND THEIR INPUT FROM NIGRAL DA+ NEURONS. E.J. Karle, K.D. Anderson and A. Reiner. Dept. of Anatomy & Neurobiology, Univ. of Tennessee, Memphis, TN 38163.

Considerable information is available regarding the synaptic inputs to medium spiny striatal neurons in general. However, little is specifically known regarding the inputs to substance P-containing (SP+) spiny striatal neurons, since these neurons label poorly immunohistochemically in mammals. To overcome this limitation, we have used immunohistochemistry to investigate this issue in pigeons, in which SP+ striatal neurons and their dendrites can be readily labeled.

EM double-labeling techniques were employed, using immunogold labeling for dopaminergic (DA+) terminals (labeled with anti-tyrosine hydroxylase) and PAP/DAB labeling for SP+ neurons. Consistent with the observed abundance of SP+ striatal neurons at the LM level, SP+ cell bodies and their dendrites were abundant at the EM level. The distal portions of these dendrites were spine-laden. These spines typically received asymmetric synaptic contacts at their tips from unlabeled terminals (presumably of cortical or thalamic origin). DA+ nigral terminals also made synaptic contacts and appositions with the SP+ neurons. The SP+ neurons also received synaptic input from SP+ terminals and from numerous unlabeled terminals whose origin is uncertain. In addition, SP+ terminals made synaptic contact with numerous unlabeled neuronal targets, including "pallidal" type dendrites that were densely coated with terminals.

These results provide direct evidence that the same major qualitative types of synaptic input to striatal medium spiny neurons are also received by SP+ striatal neurons. Supported by NS-19620, 3P01NS-26473 (A.R.) & Univ. of Tenn. Neurosci. Ctr. of Excellence (K.D.A.)

505.11

SELECTIVE DISTRIBUTION OF TYROSINE HYDROXYLASE IMMUNOREACTIVE AFFERENTS RELATIVE TO MEDIUM SPINY NEURONS IN CAUDATE PUTAMEN AND NUCLEUS ACCUMBENS OF THE RAT: A GOLGI EM STUDY. D.S. Zahm. Dept. of Anat. and Neurobiol., St. Louis Univ. Sch. Med., St. Louis, MO 63104.

Since tyrosine hydroxylase (TH) immunoreactive (IR) axospinous contacts are significantly more prevalent in the caudate-putamen and accumbal core than in the accumbal shell (Zahm and Haycock, Soc. Neurosci. Abst. 15:905), we have now addressed whether striatal medium spiny neurons in the nucleus accumbens are contacted by fewer numbers of TH-IR boutons than their counterparts in the caudate-putamen. Following immunoperoxidase demonstration of TH-IR boutons in 50μm vibratome sections, a section Golgi protocol was used to impregnate medium densely spined neurons. The positions of impregnated neurons relative to the boundaries of caudate-putamen, accumbal core or accumbal shell were established by comparison with adjacent sections processed to display substance P or 28kD calcium binding protein immunoreactivities. The numbers of immunolabeled profiles contacting profiles of identified, impregnated dendrites were expressed as a function of the summed lengths of the limiting membranes of such dendrites appearing within the zone of antibody penetration. While the word contact as used here implies only membrane apposition, punctate symmetrical synaptic specializations were occasionally observed. The analysis, which disregarded patch/matrix distinctions, revealed that medium spiny neurons in the accumbal shell are contacted over 50% less frequently by TH-IR boutons than are those in the caudate putamen and accumbal core, although the density of immunolabeled boutons was about the same in the three structures. Supported by USPHS grant NIH-23809.

505.12

MORPHOLOGICAL DIVERSITY OF TACHYKININ - PRODUCING NEURONS IN ADULT FELINE NEOSTRIATUM. M.K. Boylan and R.S. Fisher. Mental Retardation Research Center and Department of Anatomy & Cell Biology, UCLA, Los Angeles, CA 90024.

Neurons in the neostriatum produce and distribute tachykinin peptides (TKs) to all areas of the basal ganglia. However, the morphological identity of these TK-producing neurons remains controversial. In order to directly test the hypothesis that a morphologically diverse population of cells are the source of TKs in the adult neostriatum, we double labeled individual neurons with combined TK immunohistochemistry and a single slice Golgi/gold-toning procedure (to reveal somatodendritic morphology).

The majority of resulting double labeled neurons were of the medium spiny type I class, with spine-free cell bodies/proximal dendrites, densely spiny dendrites and an axon emerging from the cell body. Less frequently, medium spiny type II neurons were observed to be TK-positive. These cells had somatic and/or proximal dendritic spines, an axon emerging from dendritic origins and spiny dendrites. They were observed in all areas of the neostriatum. On occasion, medium and large sparsely spiny and aspiny cells were double labeled.

These results are the first direct demonstration that medium spiny type II and large and medium aspiny neurons are a subset of TK-producing cells in the adult neostriatum. The diversity of cell classes that produce TKs and other neurotransmitters, such as GABA, in the adult neostriatum indicates that neurotransmitter identity is not strictly associated with or limited by neuronal morphology. Supported by USPHS Grants NS 24596 and HD 05958.

505.13

REGULATION OF GAP JUNCTION PROTEINS IN NEOSTRIATUM: EXPRESSION OF CONNEXIN-32 AFTER SUBSTANTIA NIGRA DESTRUCTION. R. Fisher, M.S. Levine, N.A. Buchwald, P. Micevych and G. Zampighi. Departments of Psychiatry and Anatomy, Mental Retardation Research Center and Brain Research Institute, UCLA School of Medicine, Los Angeles, CA 90024 U.S.A.

Substantia nigra lesions augment dye-coupling between neurons in neostriatum. Our object was to determine if persistent accumulations of gap junction proteins and their encoding mRNAs contribute to this effect. Unilateral electrolytic lesions of substantia nigra in adult rats caused dopamine depletion after 14 days(decreased tyrosine hydroxylase content in neostriatum ipsilateral to lesions). As shown by in situ hybridization, mRNA encoding connexin 32 (C-32), the 32 kD hepatocyte-specific gap junctional protein, increased in neurons of dopamine-depleted neostriatum. As shown by immunohistochemistry, C-32 protein also increased in neurons of dopamine-depleted neostriatum. Both markers occurred at low levels in "intact" neostriatum of lesioned cases (5) and normal cases (3). We conclude that: 1) Dopamine may regulate gap junctions in neostriatum since loss of nigrostriatal input leads to supranormal accumulations of C-32 mRNA and protein in association with enhanced neuronal dye-coupling. 2) Gap junctions occur at low levels in intact neostriatum because C-32 protein and mRNA are detectable but barely apparent in association with low incidence of neuronal dye-coupling. Significant and progressive up-regulation of neostriatal neuronal coupling is predicted during the course of nigrostriatal dopaminergic denervation in Parkinson's disease. Supported by USPHS NS24596 and HD05958.

505.14

EXTRASTRIATAL GABAERGIC AND CHOLINERGIC INPUTS TO THE NEOSTRIATUM. T. Duong and R.S. Fisher. UCLA Mental Retardation Research Center and Department of Anatomy, Los Angeles, CA 90024.

It is widely believed that sources of GABAergic and cholinergic innervation of the neostriatum are local circuit neurons. In this study, the hypothesis that GABAergic and cholinergic neostriatal inputs originate, in part, from extrastriatal sites in the basal ganglia has been examined. We used double labeling means for detecting connectivity and transmitter molecular markers. Adult cats were injected unilaterally in the head of the caudate nucleus with the retrograde tracer wheat germ agglutinin bound to inactivated horseradish peroxidase and combined to colloidal gold. Brain sections were processed for simultaneous demonstration of the retrograde tracer and immunohistochemical labeling of choline acetyltransferase (ChAt) and glutamic acid decarboxylase (GAD). Results reveal that unilateral ChAt-immunoreactive (ChAt-ir) neurons located dorsal to the globus pallidus provide an afferent input to the head of the caudate nucleus. These ChAt-ir neurons (about 100-150 cells / case) are fusiform to multipolar and distributed at the level of and rostral to the anterior commissure. A smaller number (about 50-100) of GAD-ir neurons from this peripallidal region and from the globus pallidus itself also contibute to the pallidostriatal input. These pallidal and peripallidal projections are undecussated. In the mesencephalon, unilateral GAD-ir neurons located in the reticulate zone and in the medial portion of the compact zone of the substantia nigra form a small component of the nigrostriatal pathway. The number of contralateral GAD-ir neurons retrogradely labeled was few, if any. **These results provide morphological evidence of extrastriatal accessory GABAergic and cholinergic afferents ascending from basal ganglia regions.** This represents a first clear demonstration of transmitter diversity in these connections and may suggest transmitter variation of internal basal ganglia feedback circuits. Supported by HD07032, HDO5958 and NS24596.

505.15

DIFFERENTIAL ORGANIZATION OF INPUTS TO NEOSTRIATUM FROM PAIRED PRE- AND POST-CENTRAL CORTICAL AREAS. M. FOTUHI, V.E. KOLIATSOS, G.E. ALEXANDER, M.R. DELONG. Depts. Neurology and Neuroscience, Johns Hopkins Univ. Sch. Med.,Baltimore,MD 21205.

We have previously shown that pre- and post-central arm areas of the primary motor cortex (M1) and somatosensory cortex (S1) project to overlapping regions of putamen (Fotuhi et al. SN Abst. 15:285, 1989). In the present double anterograde study in green monkeys (using WGA-HRP and tritiated amino acids), we examined the relationship of terminal fields from the arm regions of another pair of interconnected cortical areas, the supplementary motor area (SMA) and area 5.

SMA terminals overlapped with the area 5 injection site, and vice versa. Striatal terminal fields of both areas extended over a long rostrocaudal domain of the caudate and putamen, with SMA terminals covering the more medial regions. In contrast to overlapping terminal domains of M1 and S1 projections, SMA terminal fields were segregated from, and complementary to those of area 5.

These results suggest a more complex and varied organization of corticostriatal projections from interconnected pre- and post-central areas than was predicted by our initial study.

505.16

ORGANIZATION OF MEDIAL PREFRONTAL CORTICAL PROJECTIONS TO THE VENTRAL STRIATUM. M. C. Lee and A. Y. Deutch. Departments of Psychiatry and Pharmacology, Yale University School of Medicine, New Haven, CT 06510.

Neurons in the medial prefrontal cortex (PFC) project to both the dorsal striatum (DS) and ventral striatum (VS). We have examined the organization of mPFC projections to the VS.

Anterograde tracer studies revealed that ventral mPFC sectors most heavily contribute to VS projections. PHA-L deposits into the infralimbic cortex resulted in the most selective VS labelling (without concomitant extensive labeling of fibers in the medial aspects of the DS). Ventral prelimbic cortex PHA-L injections also labeled the VS. More rostral injections of the medial orbital cortex did not result in significant VS labeling. PHA-L fibers ventromedial to the nucleus accumbens interdigitated with clusters of dense substance P-like immunoreactive fibers, i.e., typically avoided ventral pallidal sectors.

Consistent with the anterograde data, dual retrograde tracer deposits into the VS and DS revealed that the majority of mPFC neurons did not collateralize to innervate both the VS and DS, but originated from different neurons, typically in different mPFC sectors. DS projecting neurons typically were seen in the superficial aspects of layer V, whereas VS efferents originated from deep layer V.

These findings provide an anatomical substrate for recent biochemical data from our laboratory which indicate that removal of corticofugal projection neurons from tonic dopaminergic inhibition selectively enhances the responsiveness of the VS (but not the DS) dopaminergic innervations to pharmacological and environmental challenges.

Supported by grant MH-45124 and by grants from the Scottish Rite Schizophrenia Research Program and the American Parkinson Disease Association.

505.17

DUAL-TRACER COMPARISON OF THE CORTICOSTRIATAL PROJECTIONS OF THE FRONTAL EYE FIELD AND THE SUPPLEMENTARY EYE FIELD IN THE PRIMATE. H.B. Parthasarathy[1], J.D. Schall[2], A.M. Graybiel[1]. [1]Dept. of Brain and Cognitive Sciences, M.I.T., Cambridge, MA 02139. [2]Dept. of Psychology, Vanderbilt Univ., Nashville, TN 37240.

The supplementary eye field (SEF) and the frontal eye field (FEF) are two regionally distinct but interconnected areas of the frontal lobe involved in the control of saccadic eye movements. Microstimulation of FEF elicts vector-coded saccades, whereas evidence links SEF to saccades whose amplitude and direction are eye-position dependent. Both of these cortical eye fields send projections to the striatum, another region known to process visual and oculomotor information. In the experiments described here, we asked whether the SEF and FEF convey their oculomotor representations to the same or to different striatal sites.

We compared the corticostriatal projections of the two eye fields in 5 cynomolgous monkeys. Guided by responses to cortical microstimulation, we placed either of the two anterograde tracers, [^{35}S]-methionine or horseradish peroxidase - wheat germ agglutinin (HRP-WGA), into each field. Serial-section analysis of the striatum demostrated a patchy projection to the striatal matrix from each of these areas that is restricted primarily to the body of the caudate nucleus and the cell bridges between the caudate nucleus and putamen. The projections were often interdigitated, but they were largely non-overlapping.

The striatum exerts critical effects on the nigrotectal control system that modulates activity in the superior colliculus. Our results indicate that there may be separate corticostriatal circuits subserving this control in the basal ganglia. Supported by NIH grant EY02866.

505.18

PROPRIOCEPTION AND THE STRIATUM: PRIMATE SOMATOSENSORY CORTICAL AREA 3A PROJECTS MORE BROADLY TO THE STRIATUM THAN DO AREAS 3B OR 1. A. W. Flaherty, A. M. Graybiel. Dept. of Brain and Cognitive Sciences, M.I.T., Cambridge, MA 02139.

To quantify the relative contributions of projections from somatosensory cortical areas to the striatum, we have calculated how relative corticostriatal magnification factor varies with cortical area and body part representation.

In 15 squirrel monkeys we made 25 injections of anterograde tracers ([^{35}S]-methionine or HRP-WGA) into the mouth (n=8), hand (n=10), and foot (n=7) representations of area 3a (n=6), area 3b (n=13), and area 1 (n=6). Injection sites were guided by multiunit recording of somatosensory receptive fields. Cross-sectional areas of labeled tissue were measured on a computer image analyzer from 40 um sections taken 480 um apart. We defined the corticostriatal magnification factor (M) for a cortical site as the ratio of the volume of labeled striatum to the volume of the injection site in cortical layers III through V.

Three-way analysis of variance showed that neither the body part representation injected nor the tracer had a significant effect on M, whereas the cortical area injected had a very significant effect ($p<0.005$). The projection from area 3a to the striatum ($M=6.30\pm0.93$) was about twice as broad as those from area 3b ($M=3.17\pm0.34$) and area 1 ($M=2.19\pm0.27$). (All values are means ± SEM.)

This result is of interest because area 3a represents primarily deep (muscle spindle) receptors, and may be more important for position sense than are areas 3b and 1, which represent primarily cutaneous sensation. The greater corticostriatal magnification of area 3a thus supports the view that the striatum is more concerned with posture and movement control than with fine touch.

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505.19

FUNCTIONAL MAPPING OF RAT STRIATUM WITH ^{14}C DEOXYGLUCOSE: SOMATOTOPY, SENSORY-MOTOR INTEGRATION, AND EVIDENCE FOR RIGHT/LEFT SEGREGATION OF INFORMATION. Lucy L. Brown, Manuel F. Gonzalez, and Frank R. Sharp. Albert Einstein College of Medicine, Bronx NY 10464.

^{14}C deoxyglucose autoradiography was used with electrical stimulation of the cortex to map corticostriate fields of activation. Awake animals (n=23) were stimulated in forelimb MI cortex, vibrissae MI, vibrissae SI and hindlimb sensorimotor cortex. Cortical and striatal activation extended several mm anterior-posterior. Image analysis was used to define and localize activated striatal regions. Segregation of forelimb, vibrissae and hindlimb activated regions was significant in striatum. However, vibrissae sensory and motor cortex stimulation resulted in extensive overlap of activation in ipsilateral striatum, suggesting extensive sensorimotor integration. Of potential great interest is that activation contralateral to the stimulation side was .4 mm ventral to the ipsilateral activation, suggesting a place code for ipsilateral and contralateral (left/right) information in the striatum. The results confirm a definite somatotopy in striatum, but with widespread fields, especially vibrissae.

BASAL GANGLIA AND THALAMUS VIII

506.1

DISTRIBUTION OF BASIC FIBROBLAST GROWTH FACTOR IN THE ADULT RAT STRIATUM. Rosalinda C. Roberts, Cornelio G. Caday, Seth P. Finklestein and Marian DiFiglia. Massachusetts General Hospital, Boston MA. 02114

In neostriatal cultures, basic fibroblast growth factor (bFGF) enhances neuron survival and protects striatal neurons from glutamate toxicity (Freese et al., this meeting). Although bFGF has been localized to a number of CNS areas in adult brain little is known about its distribution in the neostriatum. In the present study, bFGF immunoreactivity was localized in the adult rat striatum. bFGF positive neurons were abundant throughout the striatum, with only small pockets of tissue lacking labeled cells. Many medium and large neurons were labeled, however not all cells in each subcategory were positive. At the EM level immunoreactivity, visualized with both DAB and colloidal gold, was deposited in patches throughout the cytoplasm of somata and dendrites. Reaction product was most often associated with neurofilaments and microtubules. Little or no label was found in glia or axon terminals, however bFGF positive axon initial segments were occasionally seen. Thus, bFGF may be an intra-neuronal growth factor in the mature striatum; its subcellular distribution suggests that it is not actively secreted from striatal neurons. NIH grant NS16367 to MD.

506.2

EFFECTS OF CORTICAL LESION BY THERMOCOAGULATION ON STRIATAL GENE EXPRESSION IN THE RAT : II SOMATOSTATINERGIC INTERNEURONS. P.Salin and M-F. Chesselet Dept. of Pharmacology U. of Pennsylvania Philadelphia, PA.

The cerebral cortex projects massively to the dorso-lateral part of caudate putamen (striatum), a structure primarily involved in the control of movement and cognition. A subset of cortico-striatal excitatory inputs has been shown to terminate upon a category of medium-sized aspiny interneurons expressing both neuropeptide Y (NPY) and somatostatin (SOM). Previous immunohistochemical studies have shown that the number of neurons stained for NPY and SOM increase after unilateral cortical lesion in the rat (Kerkerian et al, Eur. J .Neurosci. 2, 1990 ; Salin et al Brain Res. in press). Our goal was to determine whether striatal NPY and SOM gene expression was affected after cortical deafferentation. Fronto-parietal cortex was lesioned in rat by superficial thermocoagulation. Lesioned and control rats were sacrificed 5 or 21 days after lesion. The brains were cut on a cryostat, (10um-thick coronal sections) and processed for in situ hybridization histochemistry using a 35S radiolabeled cRNA probe. The number of labeled cells per striatal area and the intensity of labeling per individual neuron (number of pixels occupied by silver grains) were measured with an image analysis system (Morphon). In lesioned rats, the number of cells containing NPY and SOM mRNA and the intensity of labeling per individual neuron were significantly increased in the dorso-lateral striatum of both hemispheres. The parallel increase of the mRNA and peptide levels suggest that the lesion of the cerebral cortex by thermocoagulation result in an activation of NPY/SOM striatal interneurons. Supported by BNS 86-16841.

506.3

EFFECTS OF CORTICAL LESION ON STRIATAL GENE EXPRESSION IN THE RAT: I. EFFERENT NEURONS. M-F Chesselet and P. Salin. Dept. of Pharmacol. U. of Penn. Philadelphia,

Previous studies (Uhl et al. J.Neurosci.'88) have shown a decrease in striatal enkephalin (Enk) mRNA levels after cortical lesions in rats. We have further investigated the effect of cortical lesions on the levels of mRNA encoding glutamic acid decarboxylase (GAD), Enk and substance P (SP), and the corresponding peptides in the basal ganglia. Fronto-parietal cortex was lesioned unilaterally in rats by thermocoagulation and the animals were sacrificed 5, 21 and 90 days after the lesion. Cryostat-cut sections (10um) were processed either for immunohistochemistry with 125I labeled secondary antibodies or for in situ hybridization histochemistry with 35S radiolabeled cRNA probes. Sections were exposed to X ray film and the optical density of the autoradiographic signal measured. No striatal atrophy was observed at any time after the lesion. SP and Enk immunoreactivity increased in the internal (entopeduncular nucleus) and external (globus pallidus) pallidum respectively. This correlates with data on human cortical diseases or injuries (Bouras et al Neurosci. Abst. 15 861 '89). Enk and GAD mRNA levels increased in the striatum at all survival times but SP mRNA increased only at 5 days post-lesion. The results suggest a paradoxical increase in the synthesis of striatal efferent neurotransmitters after disruption of excitatory cortical pathways The discrepancy observed between ours results (by thermocoagulation lesion) and data of Uhl et al. (by aspiration lesion) could be explain by differential involvement of compensatory post-lesional processes. The prolonged and marked effects observed on Enk containing-neurons suggest that they may be a preferential target of cortico-striatal inputs. Sup by BNS 86-16841.

506.4

EFFECTS OF CLOZAPINE AND HALOPERIDOL ON STRIATO-PALLIDAL NEURONS AS REVEALED BY IN SITU HYBRIDIZATION HISTOCHEMISTRY (ISHH) IN THE RAT. M. Mercugliano and M-F. Chesselet, Dept. of Pharmacology, Univ. of Pennsylvania, Philadelphia, PA.19104

We have shown that clozapine and haloperidol have differential effects on the mRNA encoding glutamic acid decarboxylase (GAD) in the globus pallidus (GP) and entopeduncular nucleus (EP) of the rat. Clozapine's unique ability to increase GAD in the GP is likely related to its lack of motor side effects[1]. The GP and EP receive separate inputs from the striatum which contain enkephalin (ENK) and substance P (SP) respectively. We have measured the mRNAs for these peptides with ISHH in the striata of rats treated for 28 days with either haloperidol (1mg/kg/day), clozapine (20 mg/kg/day), or vehicle in order to assess the role of the striatal-GP pathway on clozapine's effect in the GP. ISHH was performed on cryostat-cut sections, with cRNA probes for ENK (S. Sabol, NIMH) and SP (H-U. Affolter, Lofstrand). Differences in labelling intensity were quantified with optical density measurements of film autoradiographs. While both drugs decreased labelling for SP mRNA.in the striatum, clozapine, but not haloperidol, decreased labelling for ENK mRNA in this area. The results show that, under these conditions, the two drugs similarly affect SP gene expression in striato-EP neurons, whereas only clozapine affects ENK gene expression in striato-GP neurons. This suggests that the increase in GAD mRNA in the GP after clozapine treatment is due to decreased activity of striato-GP neurons. Supp. by MH44894, ICI America, Inc. and MH14654 (M.M.)

1 Mercugliano, M., Chesselet, M-F., Saller, C., Salama, A. and U'Prichard, D.(1989) *Neurosci. Abstr.* **15**, 911.

506.5

EFFECT OF 6-HYDROXYDOPAMINE (6-OHDA) LESIONS OF THE SUBSTANTIA NIGRA ON THE LEVELS OF GLUTAMIC ACID DECARBOXYLASE (GAD) AND PREPROSOMATOSTATIN (SOM) mRNAs IN THE RAT PALLIDUM. Soghomonian J-J and Chesselet M-F, Dept . of Pharmacology, U. of Pennsylvania, Philadelphia, PA

...The role of the dopaminergic nigro-striatal pathway in the regulation of GAD and SOM mRNAs in neurons of the pallidum was investigated by in situ hybridization histochemistry (ISHH). Adult rats received an unilateral injection of 6-OHDA (6 µg in 2 µl) in the substantia nigra. After 7 days, animals were tested for contralateral turning with a single injection of apomorphine and sacrified two weeks later. The brains were frozen and cryostat-cut (10µm-thick). Sections were processed for ISHH with a 35S-radiolabeled cRNA for GAD (A. Tobin) or SOM (G. Goldman). In the globus pallidus of 6-OHDA-treated rats, the number of labeled cells was higher (29 ± 2.9 cells per mm^2) on the side ipsilateral to the lesion as compared to the contralateral side (14 ± 2.8) and to controls (20 ± 2.8). The intensity of labeling per cell was also higher in the globus pallidus ipsilateral to the lesion. In the entopeduncular nucleus of 6-OHDA-treated rats, the number of GAD-labeled cells was higher on the side ipsilateral to the lesion (17.5 ± 2.2) whereas the contralateral side had a number of labeled cells similar to controls (9.9 ± 1.5). By contrast, there was a massive increase (3-4 time) in the number of SOM-labeled cells, and intensity of labeling per cell, on the entopeduncular nucleus ipsilateral to the lesion (as compared to contralateral or control). These results suggest that removal of the dopaminergic neurons of the substantia nigra increases the expression of GAD mRNA in both pallidal segments in the rat and affects dramatically the expression of SOM mRNA in the entopeduncular nucleus. Supp by BNS 86-16841 and MH44894 and the PMAF (JJS).

506.6

REGULATION OF NEUROPEPTIDE EXPRESSION IN THE NUCLEUS ACCUMBENS OF THE RAT.

P.Voorn, C.R.Gerfen, Lab of Cell Biology, NIMH, Bethesda, MD 20892. Dept. Anatomy, Vrije Universiteit, Amsterdam, the Netherlands.

Enkephalin (ENK)- and substance P (SP)-immunoreactivity (IR) patterns in the nucleus accumbens (NA) are heterogeneous, showing areas of heavy, moderate or light immunostaining. Combined immunocytochemistry (ICC) and in situ hybridization histochemistry (ISHH) of ENK- and SP-mRNA with oligodeoxynucleotides showed that the heterogeneous IR-patterns in the shell region, but not in the core region of the NA can be recognized also in the distribution of ENK- and SP-mRNA. In the caudate-putamen decreased dopaminergic neurotransmission or lesion of neocortical inputs are known to cause dramatic alterations in peptide- and mRNA levels for ENK and SP. The present study focusses on possible regulation of ENK- and SP synthesis in the NA by dopaminergic and/or allocortical inputs from the hippocampus. Quantitative ISH and ICC were used to examine changes in the levels of ENK- and SP-mRNA after unilateral lesions of the dopaminergic fibers in the medial forebrain bundle with 6-OHDA and/or transsection of the fornix. Results show that 10-14 days after 6-OHDA-lesion the level of expression of ENK is increased and that of SP is decreased on the lesioned side compared to respective levels on the non-lesioned side. Dopaminergic regulation may act through direct inputs to the ENK- or SP-containing cells, but may also be relayed through cholinergic interneurons. A dopamine-acetylcholine interaction was studied at the morphological level by employing ISHH for the D2 receptor (Bunzow et al., Nature 336, 1988) and ICC for choline-acetyltransferase. Results show that cholinergic neurons in the caudate-putamen express the D2 receptor, whereas in the NA not all cholinergic neurons appear to express the D2 receptor.

506.7

COCAINE AND AMPHETAMINE INDUCE THE IMMEDIATE-EARLY GENE C-FOS IN STRIOSOME AND MATRIX COMPARTMENTS OF THE STRIATUM. A.M. Graybiel[1], R. Moratalla[1], H.A. Robertson[2] and M.R. Peterson.[2] [1]Dept. of Brain and Cognitive Sciences, M.I.T., Cambridge MA 02139 &[2]Dept of Pharmacology, Dalhousie Univ., Halifax, Nova Scotia.

Cocaine and amphetamine are psychomotor stimulant drugs that produce dramatic long-term changes in behavior. Many of these effects are thought to be mediated by dopaminergic mesostriatal systems. Here we tested the possibility that these drugs activate immediate early genes (IEGs) in the striatum. Adult rats were treated with intraperitoneal amphetamine (5 mg/kg), cocaine (25 mg/kg), or saline, and were perfused after 45 min- 6 hr. Sections through the striatum were stained for fos-like immunoreactivity and for calbindin$_{28k}$-like immunoreactivity to demonstrate striosome/matrix architecture.

Amphetamine and cocaine, but not saline, induced highly specific patterns of c-fos expression in dorsal and ventral striatum. Immunoreactivity appeared in nuclei of medium-sized striatal neurons by 45 min-1 hr, and declined by 6 hr. Cocaine induced c-fos both in striosomes and in matrix. By contrast, amphetamine induced a striking striosome-predominant pattern in the rostral caudoputamen, with more generalized induction farther caudally. In situ hybridization with oligonucleotide probes established that the induction involved c-fos transcription. Induction of c-fos-like immunoreactivity by each drug was blocked by pretreatment with the D1 dopamine antagonist SCH233390 (0.5 mg/kg), but only cocaine-elicited induction was blocked by reserpine (10 mg/kg).

We conclude that some of the physiological and behavioral effects of psychomotor stimulant drugs may depend on selective patterns of gene transcription in striosomes and matrix. Supported by The Seaver Institute, NIH NS 25529, The United Parkinson Foundation, NARSAD and MRC of Canada.

506.8

ACTIVATION OF RAT STRIATAL c-FOS BY DIRECT INFUSION OF DOPAMINERGIC AGONISTS AND FORSKOLIN. H.A Robertson[1], A.M. Graybiel[2] and M.L. Paul[1]. [1]Dept. of Pharmacology, Faculty of Medicine, Dalhousie University, Halifax, N.S., Canada B3H 4H7 and [2]Dept. Brain & Cog. Sci., MIT, Cambridge, MA 02139, U.S.A.

Recently we demonstrated that systemic injections of D1-selective dopamine agonists activate the immediate-early gene c-fos in the caudate-putamen and nucleus accumbens ipsilateral to a 6-OHDA lesion (Robertson, H.A. et al, Brain Res. 503:346, 1989). To characterize further this response, we have now investigated the effects of direct infusion of agents into the striatum in naive rats, in rats with unilateral 6-OHDA lesions and in rats pretreated with the biogenic amine-depleting drug reserpine. Drugs were slowly infused under pentobarbital anesthesia, and two hours later, rats were perfused for immunohistochemical localization of c-fos protein.

The D1-selective agonists SKF-38393 and CY-208243 produced marked c-fos activation in 6-OHDA and reserpine-treated rats but had no effect in naive rats. This response was attenuated by the D1 antagonist SCH-23390 (0.5 mg/kg, i.p.). Forskolin produced c-fos activation in both naive and treated animals, and this was unaffected by SCH-23390. In all animals, c-fos immunoreactivity was confined to the striatum.

In agreement with our earlier studies, the D2-selective agonist LY-171555 did not induce c-fos activation when infused directly into the striatum. These results suggest that the effect of the D1 agonists is direct and may be mediated by dopamine-sensitive adenylate cyclase.

(Supported by MRC of Canada and Javits NIH NS 25529).

506.9

THE EFFECTS OF ACUTE ANTIPSYCHOTIC DRUG TREATMENT ON MONOAMINE SYSTEMS IN THE NUCLEUS ACCUMBENS CORE AND SHELL. D. A. Cameron and A. Y. Deutch. Departments of Psychiatry and Pharmacology, Yale University School of Medicine, New Haven, CT 06510.

Recent anatomical studies indicate that the nucleus accumbens (NAS) can be differentiated into core (NAS_C) and shell (NAS_S) regions. The NAS_C and NAS_S have distinct afferents and efferents, and may subserve different functions. We have examined the consequences of acute antipsychotic drug (1.0 mg/kg haloperidol and 12.5 mg/kg clozapine) treatment on NAS monoamine systems.

Basal dopamine (DA) content and turnover in the two NAS regions did not significantly differ. However, serotonin (5-HT) concentration was lower, and 5-HIAA concentration and 5-HT turnover higher, in the NAS core than in the shell.

Haloperidol and clozapine increased DA turnover in both the NAS_C and NAS_S; haloperidol augmented dopamine turnover to a significantly greater degree than did clozapine. DA turnover was not differentially augmented between the two NAS regions. In contrast, 5-HT turnover was enhanced by both haloperidol and clozapine in the NAS shell only; serotonin turnover was not increased in the NAS_C. Haloperidol and clozapine augmented NAS_S 5-HT turnover by the same magnitude.

These data indicate that the NAS core and NAS shell can be pharmacologically dissociated. These data thus complement anatomical data differentiating the core and shell regions of the NAS, and suggest that functional differences may soon be uncovered.

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506.10

Excitatory Amino Acid Receptors in Rat Basal Ganglia. RL Albin, RL Makowiec, Z Hollingsworth, LS Dure, JB Penney, AB Young. Dept. of Neurol., Univ. of Michigan, Ann Arbor, MI 48109

Excitatory amino acids (EAAs) are the neurotransmitters of numerous basal ganglia afferents and circuits. Quantitative receptor autoradiography was used to determine the distribution of EAA receptors in rat basal ganglia. NMDA, AMPA, KA, and metabotropic (MET) receptors, and the non-NMDA-KA-Quisqualate glutamate binding site (NNKQG) were densest in striatal regions. AMPA, MET, and NMDA receptors were denser in ventral than dorsal striatum. KA and NNKQG were uniformly dense in both ventral and dorsal striatum. All receptors had lower density in GP, VP, EP, STN, SN, and VTA. Receptor density was invariably higher in VP than GP with VP having relatively high density of AMPA, NNKQG, and KA binding. AMPA and MET receptors were relatively dense in STN. NNKQG binding was relatively high in SN. Inhomogeneous EAA receptor distribution indicates heterogeneous behavior of EAA neurotransmission within the basal ganglia. Supported by NS01300, NS19613.

506.11

NMDA RECEPTOR ACTIVATION IN THE NEOSTRIATUM INCREASES FOS SELECTIVELY IN MEDIUM SIZED NEURONS BUT NOT LARGE CELLS. N. Aronin and M. DiFiglia, Dept. of Medicine, Univ. of Mass. Med. Sch., Worcester, MA 02160, and Dept. of Neurology, Mass. General Hosp., Boston, MA 02114.

We sought to identify which neostriatal cells are responsive to NMDA receptor activation. The NMDA agonist quinolinic acid (QA, 10ug in 0.5ul) was injected into the rat caudate (n=10); 2h later immunohistochemistry was performed using affinity-purified antisera against the 127-154 fragment of Fos, a candidate effector of gene transcription. Results: QA injection increased nuclear labeling of Fos throughout the neostriatum; CSF infused into the contralateral caudate produced sparse labeling of nuclei only at the site of injection. Co-injection of QA with the NMDA receptor antagonist APV prevented Fos expression. Fos was localized predominately to medium sized neurons, including cells reactive for NADPH diaphorase; most large neurons were devoid of nuclear labeling. Ultrastructural examination verified that Fos was confined to the nuclei of medium sized neurons with unindented and indented nuclei; reaction product was distributed unevenly in the karyoplasm and notably in patches along the inner face of the nuclear membrane. Implications: Medium sized spiny and aspiny neurons have a high density of NMDA receptors and activation of NMDA receptors in these neurons contributes to the regulation of c-fos gene expression. Supported by the NSF and NIH.

506.12

Muscarinic Receptors Modulate Apomorphine Induction of Dynorphin in Striatal Patch Neurons J.B.Daunais and J.F. McGinty Department of Anatomy and Cell Biology East Carolina University School of Medicine, Greenville N.C. 27858

Previous studies have shown that repeated injections of the dopamine (DA) agonist, apomorphine (APO), increase dynorphin immunoreactivity (DYNir) and mRNA in striatal patch neurons (Li et al. JPET 246:403, Gerfen, C.R., McGinty, J.F. and Young, S.W. submitted). Stimulation of DA receptors also leads to decreased acetylcholine (Ach) turnover in striatal neurons. This study was conducted to investigate whether cholinergic tone affects the APO-induced DYN increase in striatonigral neurons.

The muscarinic agonist, oxotremorine 0.46 mg/kg (OXO), or muscarinic antagonist, scopolamine 10.0 mg/kg (SCOP) was administered s.c. 30 min. prior to 5.0 mg/kg, s.c. APO or saline twice daily for seven days. Eighteen hours after the final injection, the rats were perfused with 4% buffered paraformaldehyde for immunocytochemistry (ICC). ICC analysis using an antibody to DYN 1-8 (J.S.Hong, NIEHS) demonstrated increased DYNir in striatal patches with no appreciable difference between the control group and the SCOP alone or OXO alone groups. OXO decreased, whereas SCOP augmented, the APO-induced DYNir in cells and fibers of striatal patches. These data support the conclusion that a decrease in cholinergic interneuronal activity mediates the APO-induced increase in DYN synthesis in striatal patches. Supported by DA 03982 and Sigma Xi.

BRAINSTEM SYSTEMS

507.1

DISCHARGES OF SUBSTANTIA NIGRA RETICULATA (SNR) NEURONS DURING SLEEP-WAKING STATES. S. Datta, R. Curró Dossi, D. Paré, G. Oakson* and M. Steriade, Lab. Neurophysiol., Sch. Med., Univ. Laval, Quebec, Canada.

We have recently described several categories of mesopontine cholinergic neurons related to the genesis and brainstem-thalamic transfer of ponto-geniculo-occipital (PGO) waves (Steriade et al., 1990, J. Neurosci., in press). As one of these cellular types discharged stereotyped spike bursts over a background of decreased firing rate during REM sleep, we hypothesized that these PGO-on bursts are generated by low-threshold spikes deinactivated by membrane hyperpolarization and further suggested that GABAergic SNR cells represent one of the possible sources of hyperpolarization in peribrachial (PB) neurons.

To test this hypothesis, we recorded single SNR cells in chronically-implanted, naturally sleeping cats. SNR cells were antidromically identified from the PB area and ventromedial thalamic nucleus. Quantitative data were obtained from a sample of 16 SNR cells recorded during waking (W), EEG-synchronized sleep (S), and REM sleep. Rates of spontaneous discharges were similar in W (26.3 Hz) and S (28.8 Hz), but increased significantly during REM sleep (42.8 Hz). In all states, SNR neurons discharged tonically, with absence of high-frequency bursts and quite symmetrical interspike interval histograms. Out of 16 identified SNR cells, 7 increased their tonic firing 70-100 ms prior to the thalamic PGO wave. These data suggest that a population of SNR cells may exert tonic and/or phasic inhibitory actions upon a class of PB neurons that fire PGO-on bursts crowning the low-threshold spike. Supported by MRC grant MT-3689.

507.2

SEROTONIN (5-HT) INHIBITS MESOPONTINE CHOLINERGIC NEURONS *IN VITRO*. C.S. Leonard and R. Llinás, Center for Neural Science, NYU 6 Wash. Pl. NY, NY 10003 and Dept. Physiol. & Biophys., NYU Med. Ctr. 550 First Ave. NY, NY 10016.

The effect of 5-HT on neurons of the pedunculopontine (PPT) and laterodorsal tegmental (LDT) nucleus was studied with intracellular recording methods in brain slice preparations from guinea pig and rat. Cholinergic neurons were identified physiologically and morphologically by combined intracellular injection of lucifer yellow or biocytin and histochemical staining for NADPH-diaphorase (Leonard and Llinás, '88, Soc. Neurosci. Abst. 14: 297), which selectively labels mesopontine cholinergic neurons (Vincent et al., '83, Neurosci. Letts. 43: 31-36). Focal application of 5-HT on physiologically identified cholinergic neurons (type II cells, Leonard and Llinás, '90, In: Brain Cholinergic Systems, Steriade and Biesold, Eds.) produced a transient suppression of firing and membrane hyperpolarization. Bath application of 5-HT produced a maintained hyperpolarization whose magnitude varied with 5-HT concentration (3-30μM). The hyperpolarization was unaffected by Ringer containing TTX and low Ca/ 2mM Co, indicating a direct action of 5-HT on mesopontine neurons. The hyperpolarization reversed near the potassium equilibrium potential and was accompanied by a large decrease in membrane resistance suggesting the activation of a potassium current. These results imply that mesopontine cholinergic neurons are directly inhibited by their serotonergic afferents and suggest that they become disinhibited during REM sleep when serotonergic neurons exhibit greatly reduced discharge rates. Supported by NINCDS13742 and a grant from the American Parkinson Disease Association.

507.3

LATERAL HYPOTHALAMIC PROJECTIONS TO THE PEDUNCULOPONTINE TEGMENTAL NUCLEUS AND ADJACENT MIDBRAIN TEGMENTUM IN THE RAT. T.L. Steininger and B.H. Wainer. Dept. of Pharm. and Phys. Science, Univ. of Chicago, Chicago IL 60637.

The cholinergic neurons of the pedunculopontine tegmental nucleus (PPT) are thought to modulate behavioral arousal through its widespread projections to the thalamus and brainstem reticular formation. Retrograde tracing studies suggest that this nucleus receives information from a variety of sources, including the posterior lateral hypothalamic area (LHAp). To further evaluate these putative afferents to the PPT, anterograde tracing with *phaseolus vulgaris* leukoagglutinin (PHA-L) was combined with immunocytochemical localization of choline acetyltransferase (ChAT).

Iontophoretic injections of 2.5% PHA-L were placed into the LHAp. The tissue was processed for the sequential immunolocalization of PHA-L with diaminobenzidine as the chromagen and of ChAT with benzedine dihydorchloride. Few PHA-L immunoreactive varicose fibers were seen in proximity to ChAT immunoreactive neurons in the PPT and laterodorsal tegmental nucleus (LDT). However, moderate to dense labeling was seen in the retrorubral field adjacent to the rostral PPT, in the central tegmental field adjacent to mid and caudal levels of the PPT, and in the medial parabrachial subnucleus situated caudally adjacent to the PPT. Descending projections from the LHAp may terminate mainly on noncholinergic neurons in the midbrain tegmentum. (NS 17661, MH09919, and GM 07839)

507.4

THE CLASSIFICATION OF AXON TERMINALS FROM BRAINSTEM STRUCTURES TO THE MEDIODORSAL THALAMIC NUCLEUS (MD) IN THE RAT. M.Kuroda and J.L.Price, Dept. Anat. & Neurobiol., Washington Univ. Sch. of Med., St.Louis, MO 63110.

Presynaptic terminals from the brainstem to MD were studied with the electron microscope in the rat, by means of anterograde transport of WGA-HRP. Labeled axons were seen mainly in the lateral part of MD after the injections of 1% WGA-HRP (50 nl) into the substantia nigra (SN), superior colliculus (SC), and dorsolateral tegmentum (DLT). The boutons arising from SC were small (<1 μm in diameter), formed asymmetrical synaptic contacts with small dendrites, and contained round synaptic vesicles. The axon terminals from DLT relatively large boutons (2-4 μm) with asymmetric synaptic contacts and round vesicles. The pre- and postsynaptic elements were surrounded by glial lamellae, to form glomeruli. These ultrastructural features are almost identical to the previously described boutons in the medial and central segments of MD that originate from the basal amygdaloid nucleus and the piriform cortex. In contrast, the nigral afferent terminals were medium to large in size (2-3 μm), made symmetrical synaptic junctions with dendritic shafts and occasionally with somata, and contained pleomorphic vesicles. These terminals are similar to the ventral pallidal afferents to the medial part of MD. Moreover, a combined study with GAD-immunohistochemistry and WGA-HRP labeling revealed that the terminals of nigral origin were immunoreactive for GAD.

These results suggest that the afferents from SC and DLT are excitatory, while nigral projections are inhibitory and GABAergic.

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507.5

LDT-PPT CHOLINERGIC NEURONS COLLATERALIZE TO TWO THALAMIC TARGETS. P. Shiromani, J. Velazquez-Moctezuma, and C. Floyd.* San Diego VAMC and UCSD, La Jolla, CA 92093.

Cholinergic neurons in the lateral dorsal tegmental (LDT) and pedunculo-pontine tegmental (PPT) nuclei have been shown to heavily innervate the thalamus. In order to determine whether single LDT-PPT cholinergic neurons simultaneously project to two thalamic targets, in rats, unilateral microinjections (0.1 ul) of rhodamine-conjugated microbeads were made into the central-lateral (CL) thalamic nucleus, and FITC-conjugated microbeads (0.1 ul) were injected into the lateral geniculate nucleus (LGN). Pontine sections were processed for immunohistochemical localization of choline acetyltransferase (ChAT). As previously shown by others, we found a heavier projection to the CL compared to the LGN and more neurons were labelled ipsilateral to the injection site. About 5% of LDT-PPT ChAT positive somata were found to contain both rhodamine and FITC beads indicating that some LDT-PPT cholinergic neurons project to both the CL and the LGN. Some LDT-PPT somata were non-cholinergic but projected to the two thalamic targets. Such a collateralization might be useful in the production of events related REM sleep. LDT-PPT cholinergic neurons are implicated in producing EEG desynchrony via intralaminar thalamo-cortical neurons. LDT-PPT cholinergic neurons are also implicated in producing PGO wave activity recorded in the LGN. We postulate that by projecting to two thalamic targets, a minority of the LDT-PPT cholinergic neurons influence two thalamic targets and thereby might coordinate the simultaneous occurrence of cortical desynchrony and PGO waves during REM sleep.

507.6

RAPHE-RAPHE INTERCONNECTIONS IN THE RAT DEMONSTRATED BY ANTEROGRADE LABELING WITH PHASEOLUS VULGARIS LEUCOAGGLUTININ. M.R. Park. Dept. Anatomy & Neurobiology, Univ. Tennessee, Memphis, The Health Science Center, Memphis, TN 38163.

Interconnections between raphe nuclei have long been implicated from retrograde axon tracing studies. In the present study, injections of the lectin phaseolus vulgaris leucoagglutinin (PHA-L) into various midline brainstem and mesencephalic targets produce networks of anterogradely labeled varicose axons in the dorsal raphe nucleus. This is true for structures that are immediately ventral to the dorsal raphe nucleus: median raphe, rostral linear raphe, caudal linear raphe, and a dorsal portion of the interpeduncular nucleus in which serotonin immunoreactive cell bodies are found. These structures lie along the caudal portion of an axis, extending caudally and dorsally from the medial forebrain bundle, of raphe-projecting nuclei. This is also the axis along which a major portions of afferent and efferent axons to and from the dorsal raphe nucleus, respectively, travel. Distant from this axis, PHA-L injections in raphe pontis also produce labeled terminal axonal fields in the dorsal and median raphe nuclei. Reconstruction of these axon pathways confirm the idea that reticular formation nuclei are richly interconnected.

The nature of the numerous axon varicosities remains unclarified. In common with other afferents to the dorsal raphe nucleus, such as the lateral hypothalamic area and ventral tegmental area, varicosities are often not in close apposition to 5-HT immunoreactive neurons, in double labeled material. This suggests that either the varicosities seen in PHA-L material do not all correspond to presynaptic boutons or that the predominate target for some dorsal raphe afferents is not the population of serotonergic neurons.

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507.7

EFFERENT PROJECTIONS FROM THE DORSAL RAPHE NUCLEUS. L.J. Sim and S.A. Joseph, Neuroendocrine Unit, University of Rochester, Rochester, N.Y. 14642.

The dorsal raphe nucleus (DRN) and ventrolateral periaqueductal gray (PAG) have been identified as part of a descending pain modulating pathway. Neurons in this region have also been shown to influence cardiovascular and homeostatic function and behavior, such as the expression of fear. The dorsal raphe complex contains serotonergic, catecholaminergic and peptidergic systems. In this study, we have injected the anterograde tracer phaseolus vulgaris leucoagglutinin (PHA-L) into various subdivisions and surrounding nuclei of the dorsal raphe complex to define the trajectory of fibers emanating from this region. PHA-L was iontophoresed into specific areas throughout the rostral-caudal extent of the DRN. Animals were sacrificed 10-15 days later and PHA-L was identified using nickel enhanced immunocytochemistry. PHA-L immunoreactive (-ir) fibers and terminals are identified in numerous forebrain nuclei including the nucleus accumbens, bed nucleus of the stria terminalis, septum and amygdala. PHA-L-ir fibers are also identified in diencephalic and brainstem nuclei including the paraventricular and medial thalamus, paraventricular nucleus, periventricular gray, lateral habenula, locus coeruleus, parabrachial nucleus, nucleus of the solitary tract and throughout the DRN and PAG. PHA-L-ir fibers are also found in the dorsal horn and central gray of the spinal cord. This anatomical data substantiates behavioral studies in which the DRN has been shown to modulate descending pain pathways as well as ascending autonomic and behavioral function. (Supported by USPHS DA 07232, NS 21323 and American Heart Association 87 1011.)

507.8

SINGLE UNIT ACTIVITY IN THE PARABRACHIAL REGION (PB) DURING STIMULATION OF THE AMYGDALOID CENTRAL NUCLEUS (ACE) AND PAVLOVIAN CONDITIONING IN RABBITS. J.P.Pascoe & B.S.Kapp. Dept. Psychology, Univ. Vermont, Burlington, VT 05405

The ACE is an essential part of a forebrain system that contributes to the autonomic features of emotion. The PB has reciprocal connections with the ACE and may be a relay in ascending and descending pathways between the ACE and various sensory and visceral motor areas. We have examined activity in PB neurons during stimulation of the ACE and during the Pavlovian conditioned bradycardic response.

Of 133 PB neurons, 35 were activated antidromically from the ACE (6-26 ms, $\bar{X}$=13 ms) and were located medial (14) or just lateral (21) to the brachium conjunctivum (BC). Activity in these neurons was infrequent, was decreased or not altered during conditioning, and was unaffected by pinna shock. Of 38 neurons activated orthodromically from the ACE (4-45 ms, $\bar{X}$=7 ms), 3 were located medial and 16 just lateral to the BC, and 19 were located dorsally within the mesencephalic area between the BC and the inferior colliculus. In many of these neurons a short latency (<15 ms) increase in activity occurred to presentations of both tone and pinna shock during conditioning. A respiratory rhythm was evident in the ongoing activity of 14 neurons located near the ventrolateral tip of the BC. These data suggest a convergence of sensory input and ACE output at the level of the PB, but the role of PB input to the ACE during conditioning remains to be identified. Supported by the American Heart Association and the AHA Vermont Affiliate, Inc.

507.9

BRAINSTEM PROJECTIONS FROM THE NUCLEUS OF THE SOLITARY TRACT IN THE RAT. E.T.Cunningham, Jr. and P.E. Sawchenko. The Salk Institute for Biological Studies, La Jolla, CA 92037.

Anterograde transport, retrograde transport, and immunohistochemical techniques were used to characterize the organization of brainstem projections from the nucleus of the solitary tract (NTS) in the rat. The results are as follows: 1) Nearly all subregions of the NTS give rise to an extensive set of intrinsic projections that almost invariably involve "associational" and "commissural" projections within that subregion on the ipsilateral and contralateral sides. In addition, the rostral part of the NTS gives rise to a strong input to the ventral and ventrolateral parts of the nucleus. The central subnucleus, in contrast to all other parts of the NTS, gives rise to, and receives, only sparse intrinsic inputs. 2) The ventral and ventrolateral parts of the NTS project to the ventromedial division of the trigeminal motor nucleus, to the intermediate subdivision of the facial nucleus, to the caudal ventral parts of the hypoglossal nucleus, and to the region of the nucleus ambiguus. 3) The central subnucleus gives rise to a dense, primarily ipsilateral, projection to the rostral, or compact, part of the nucleus ambiguus. 4) Most parts of the caudal, medial half of the NTS, including the commissural portion, project heavily to the dorsal motor nucleus of the vagus nerve (DMX). 5) The central gray (CG) receives projections principally from those parts of the medial and commissural NTS known to contain the A2 and C2 catecholamine cell groups. 6) The A1 and C1 catecholamine cell groups in the ventrolateral medulla receive their heaviest and most directed input from the commissural part of the NTS. 7) The parabrachial nucleus receives a dense input from virtually all parts of the NTS, with the exception of the central subnucleus. Together, these results provide evidence for topographically discrete pathways from the NTS to a number of motor and relay nuclei important in autonomic, neuroendocrine and orofacial function.

507.10

IN VITRO RAT BRAINSTEM SLICES REVEAL GUSTATORY NEURON TYPES. R.M. Bradley and R.D. Sweazey. School of Dentistry, University of Michigan, Ann Arbor, MI 48109-1078.

Using intracellular recordings and biocytin injections we are defining the intrinsic membrane properties of neurons in the gustatory zone of the solitary tract nucleus (NTS). Three neuron types were separated using current injection pulse paradigms in rat brainstem *in vitro* slice preparations. Injections of depolarizing current produced a low frequency, regular pattern of response in Type I neurons; a high frequency, regular response in Type II neurons; and a high frequency, burst-like pattern of response in Type III neurons. Furthermore, in both Type I and III neurons, preceding the depolarizing current pulse by a hyperpolarizing prepulse produced a delay in the initiation of the first spike. This delay was dependent on both the magnitude and duration of the prepulse. Preliminary reconstructions of biocytin-filled neurons indicate that these physiologically defined neuron groups also have different morphological characteristics. The presence of neuron types in the gustatory NTS suggests that these groups have different roles in processing gustatory information.

Supported by N.I.H. Grant DC00288.

507.11

ELECTROPHYSIOLOGY AND MORPHOLOGY OF CO2-SENSITIVE NEURONS IN THE DORSAL VAGAL COMPLEX STUDIED IN VITRO. J.B. Dean, E.A. Gallman, and D.E. Millhorn. Dept. of Physiology, Univ. of North Carolina, Chapel Hill, NC 27599.

We reported previously (*Exp. Brain Res.*, 76:656, 1989; *Neurosci.*, in press) that certain neurons in the nucleus tractus solitarii (NTS) and dorsal motor nucleus are depolarized inherently by hypercapnia. Additional work, reported here, using the *in vitro* slice preparation (rat) shows that CO_2-induced changes in excitability are associated with one or more of the following: decreased amplitude & duration of afterhyperpolarization, depolarization of the membrane, & decreased conductance. In one NTS neuron with slow oscillations of membrane potential, hypercapnia increased the magnitude and rate of oscillation. CO_2-induced depolarization was unaffected by 1μM tetrodotoxin, but prevented by pretreatment with 2 mM 4-aminopyridine. Lucifer Yellow filled somata of CO_2-sensitive cells were either pyramidal, oval, or fusiform as viewed transversely and produced 3-5 primary processes. Somata ranged from 28-50μm in their longest diameter. Most processes appeared to radiate towards the dorsal surface, fourth ventricle, or central canal, sometimes coming to within 20μm of these structures. Experiments are underway to determine the efferent projection of CO_2-sensitive neurons using rhodamine microspheres. (NRSA HL07398, NIH HL33831, AHA 881108, ALA)

507.12

IN VITRO STUDIES OF HYPOXIC MODULATION OF MEMBRANE PROPERTIES IN THE DORSAL VAGAL COMPLEX E.A. Gallman, J.B. Dean and D.E. Millhorn, UNC, Chapel Hill, NC 27599

The present study was undertaken to determine the direct effects of hypoxia on neurons of the dorsal vagal complex (nuc. solitary tract, dorsal motor nuc. of the vagus). Since this region is known to have diverse function (*e.g.* respiratory, cardiovascular control), we wondered if cells in this region might also exhibit diverse responses to hypoxia. Intrinsic hypoxic responses of these neurons have a direct bearing on their functioning in homeostatic control. Intracellular recordings were made in rat brain slices in a perfusion-interface chamber at 35-37°C while exposed to 95% O_2/5% CO_2. Cells were tested in hypoxia (95%N_2/5%CO_2) for 2-3 min. Some neurons tested were hyperpolarized by hypoxia with a decrease in R_N, suggesting an increased gK^+. Such neurons decreased firing rate during hypoxia. Other neurons tested were depolarized with a large decrease in R_N and decreased firing rate, suggesting a depolarizing block. Still other neurons were excited by hypoxia, depolarizing slightly and increased firing rate with little change in R_N. There were also cells which were not responsive to this brief hypoxic challenge. Thus cells within the DVC exhibit a number of responses to hypoxia which may reflect the diverse functions of this region. (USPHS Grant HL-33831)

507.13

CONNECTIONS OF THE DORSAL VAGAL COMPLEX IN THE FERRET [Mustela putorius furo]. N.L. Strominger, A.P. Knox and D.O. Carpenter. Department of Anatomy, Cell Biology and Neurobiology, Albany Medical College, Albany, NY 12208.

The connectivity of the ferret dorsal vagal complex was studied with HRP or WGA-HRP delivered via micropipette. Animals were perfused transcardially with buffered saline followed by 2.5% gluteraldehyde in 0.1 M phosphate buffer after 24-48 hours. Tissues were cut at 50um on a freezing microtome and reacted with tetramethyl benzidine. In different animals, injections were restricted to the AP, were immediately adjacent to the AP centered in the solitary nuclear complex, or involved the AP together with the adjacent tegmentum. In cases with injections limited to the AP, labeled fibers could be traced only into the adjacent solitary complex, specifically the medial and subgelatinosus subnuclei. These nuclei also contained labeled perikarya. After injections centered in the solitary complex, labeled perikarya occurred in the AP, parabrachial nucleus, paraventricular nucleus of the hypothalamus and in the substantia innominata. Efferently labeled fibers were followed to the paraventricular nucleus. We conclude that in ferrets ascending brainstem pathways from the AP relay in the solitary complex.

507.14

DISTRIBUTION OF NEUROPEPTIDES AND 5-HT IN THE FERRET BRAINSTEM. I. THE DORSAL VAGAL COMPLEX. G.E. Lucier, R. Egizii, F.M. Boissonade and K.A. Sharkey. Dept. Medical Physiology, University of Calgary, Calgary, Alberta T2N 4N1 Canada

The ferret is becoming increasingly important in neurophysiological studies. Since we are using this animal for studies of G.I. tract function, it was necessary to examine the central distribution of various neurochemicals in nucleus tractus solitarius (nTS), area postrema (AP) and dorsal motor nucleus of the vagus (DMV), [the dorsal vagal complex (DVC)]. In order to help define the boundaries of these nuclei in the ferret, the location of the DMV and hypoglossal (XII) nuclei were confirmed using retrograde tracing with HRP applied to the cervical vagus or XII nerve respectively. Frozen 40μm serial brainstem sections were cut, incubated with antibodies against substance P (SP), calcitonin gene-related peptide (CGRP), enkephalin (ENK) and serotonin (5-HT), and processed using the PAP procedure. All immunoreactivity (IR) appeared in fibers and/or terminals. In the nTS, SP-, and to a lesser extent, CGRP-IR were present in discrete areas, suggesting localization within subnuclei. Dense SP- and CGRP-IR were also found in the AP and the DMV. In contrast, ENK-IR was evenly distributed, whereas 5-HT-IR was sparse and scattered, but both were found throughout the DVC. The only IR cell bodies seen were those containing 5-HT in the DMV. Vagal nerve fibers entering the brainstem (approx. 2.0 mm rostral to obex) contained primarily CGRP-IR, to a lesser extent 5-HT-IR and occasionally SP-IR. (Supported by Canadian MRC and Alberta Heritage Foundation for Medical Research).

507.15

DISTRIBUTION OF NEUROPEPTIDES AND 5-HT IN THE FERRET BRAINSTEM. II. THE TRIGEMINAL COMPLEX. F.M. Boissonade, K.A. Sharkey, R. Egizii and G.E. Lucier. Dept. Medical Physiology, University of Calgary, Calgary, Alberta T2N 4N1 Canada.

As a basis for future neurophysiological studies in the ferret, it was necessary to examine the central distribution of various neurochemicals in the trigeminal complex: main sensory nucleus (MSN) and spinal trigeminal nucleus (spV), divided into caudalis, interpolaris and oralis. Frozen 40μm serial brainstem sections were cut, incubated with antibodies against substance P (SP), calcitonin gene-related peptide (CGRP), enkephalin (ENK) and serotonin (5-HT), and processed using the PAP procedure. In caudalis, dense SP-, CGRP- and ENK-immunoreactive fibers and terminals (IR) were present as a single band in laminae I and II, and less densely in lamina V. At ~ 800 μm caudal to obex, the central portion of the SP- and CGRP-IR band in laminae I and II disappeared and an additional band appeared along the dorsomedial border of laminae IV and V. In contrast, 5-HT-IR was present over the entire sub-nucleus with the most dense IR in laminae I and II. In caudal interpolaris, SP-, CGRP- and ENK-IR was restricted to the dorsolateral border and a ventromedial portion of the nucleus. This IR disappeared in rostral interpolaris. Sparse 5-HT-IR was present throughout interpolaris and most dense in a band along the medial border. In oralis, all substances were found in IR fibers crossing the trigeminal tract and as terminals and fibers scattered around the periphery of the nucleus, especially along the dorsomedial border. In MSN, SP-, CGRP- and ENK-IR was localized in the dorsomedial part of the nucleus and 5-HT-IR was evenly distributed throughout. (Supported by Canadian MRC and Alberta Heritage Foundation for Medical Research).

LEARNING AND MEMORY—PHARMACOLOGY: MONOAMINES

508.1

OLFACTORY BULB NOREPINEPHRINE MAY BE REQUIRED FOR EARLY OLFACTORY LEARNING. Weiquan Lin, D.A. Wilson, and R.M. Sullivan. Developmental Psychobiology Laboratory, Dept. Psychology, University of Oklahoma, Norman, OK

Norepinephrine (NE) is known to be critically involved in a variety of olfactory learning paradigms for acquisition of conditioned neurobehavioral responses. For example, in rat pups, systemic injections of NE β-receptor antagonists can block olfactory preference conditioning and its neural correlates (Sullivan et al., 1989). The present study limited NE blockade to the olfactory bulb during training, to determine the role of bulb NE in early learning.

On PN5, under cold anesthesia, pups had a cannula (30 ga) chronically implanted into one bulb and had the contralateral naris occluded. Pups were returned to the litter until PN6, when olfactory conditioning occurred. Pups were trained in one of 3 groups, PAIRED - odor paired with tactile stimulation, RANDOM, or ODOR ONLY. During a 10 min habituation period and the 10 min training session, pups had either saline or 100 μM propranolol infused into the bulb (0.1 μL/min). On PN7, pups were given a behavioral odor preference test or injected with [14C] 2-DG and exposed to the conditioned odor. Preliminary results suggest that NE antagonists limited to the bulb block acquisition of a learned behavioral odor preference. Neural correlates of this response are being examined.

508.2

DSP-4, SOCIAL HOUSING CONDITIONS AND OLFACTORY EXPERIENCE INFLUENCE NOVELTY-INDUCED ANXIETY IN RATS. C.A. Cornwell-Jones, T. Palfai*, D. Krasenbaum*, E. Eyer Jr.*, B. Clark* and K. Kinnard*. Department of Psychology, Syracuse University, Syracuse, NY 13244.

Three experiments tested the hypothesis that housing control and DSP-4 treated rats together is stressful and increases anxiety. Rats were injected s.c. with water or the norepinephrine (NE) neurotoxin DSP-4 on the day of birth, placed at weaning in bedding with either a familiar or novel odor, and observed in three different situations 11-13 days later. Rats housed in the familiar odor in mixed groups (DSP-4 and water) showed abnormally low levels of rearing in the open field, and social interaction with strange rats, indicators of anxiety. These effects were not seen in rats from mixed groups housed in the novel odor. The data suggest that exposure to a novel odor can reduce anxiety. Mixed housing in either odor elevated rats' exploratory behavior in the home cage, and also depressed frontal cortex NE levels for DSP-4-treated rats. These data suggest that mixed housing is stressful, particularly for DSP-4 treated rats.

508.3

THE EFFECTS OF ALPHA-2 ADRENERGIC DRUGS ON CORTICAL AND HIPPOCAMPAL ELECTRICAL ACTIVITY AND LEARNING/MEMORY IN RATS WITH COGNITIVE DYSFUNCTION. J. Sirviö, P. Riekkinen Jr.*, A. Valjakka*, T. Halonen* and P.J. Riekkinen. Dept. of Neurology, University of Kuopio, Finland

The present set of experiments were undertaken in order to study whether alpha-2 adrenergic agonist (guanfacine) or antagonist (atipamezole) would improve age-associated cognitive dysfunctions. We studied the effects of different doses of those drugs on cortical electrical activity (spectral electroencephalogram (EEG) the amount of high voltage spindles (HVS)) and hippocampal electrical activity as well as acquisition and retention of passive avoidance (PA) and water maze (WM) tasks. The models used were aged (24-months old) rats, fimbria-fornix (FF)- and nucleus basalis (NB)-lesioned rats. Surgery of animals (implantation of electrodes and lesions) were done under deep anesthesia. Guanfacine (Sandoz Ltd, Switzerland) was injected intraperitoneally (4 ml/kg), and atipamezole (Farmos Ltd, Finland) was injected subcutenously (0.5 ml/kg). Vehicle treated rats received saline. The most significant findings were:
1) atipamezole (3 mg/kg) decreased the number of HVS and improved the performance in PA test (testing latency) of aged rats 2) atipamezole (3 mg/kg) decreased the delta and theta power of nb-lesioned rats
3) guanfacine (0.004-0.1 mg/kg) increased the number of HVS (which were blocked by atipamezole) and theta power both in young and aged rats
4) guanfacine (0.001 mg/kg) impaired the performance of young and aged rats in WM task 5) atipamezole (1 and 3 mg/kg) increased immobility-related power of 4-8 Hz (theta) band of hippocampal EEG in both FF-lesioned and sham-operated rats
These results suggest that a selective alpha-2 antagonist may improve cognitive dysfunctions related to aging.

508.4

INVOLVEMENT OF PERIPHERAL AND CENTRAL β-NORADRENERGIC RECEPTORS IN MEMORY STORAGE. **I. B. Introini-Collison, D. Saghafi* and J.L. McGaugh. Ctr. Neurobio . Learning/Memory and Dept. Psychobiology. U.C. Irvine.**

Peripheral administration of epinephrine modulates memory in a time- and dose-dependent manner. Since epinephrine does not readily cross the blood-brain barrier, it is not likely that it exerts its effects directly in the central nervous system. The present experiments compared the effects of epinephrine and dipivefrin (DPE) on retention of an inhibitory avoidance task and a reversal visual discrimination task in mice. The most effective dose of epinephrine for inducing memory facilitation was 0.1 mg/kg. DPE, a less polar analogue of epinephrine which has a higher tendency to cross membranes, also significantly facilitated memory for both tasks when administered posttraining. The dose-response curve also followed an inverted-U shape with 0.01 mg/kg most effective. The enhancing effects of DPE on memory appear to be mediated by central β-adrenoceptors: the centrally-acting β-noradrenergic antagonist propranolol, but not the peripherally-acting β-adrenergic antagonist sotalol, blocked DPE-induced enhancement of memory. However, sotalol did prevent the effects of epinephrine on memory, suggesting a peripheral site of action for epinephrine. α-Adrenoceptors do not appear to be involved in the facilitatory effects of either DPE or epinephrine on memory: the peripherally-acting α-adrenergic blocker phentolamine did not prevent epinephrine-induced enhancement of memory, while neither phentolamine nor the centrally acting α-adrenergic blockers prazosin or yohimbine blocked the facilitatory effects of DPE on memory. These results support the view that epinephrine initiates its facilitatory effects on memory through the activation of peripheral β-noradrenergic mechanisms, while DPE exerts its effects directly in the brain.

508.5

MEMORY IMPAIRMENTS WITH MEDIAL SEPTAL MORPHINE INJECTIONS: ATTENUATION WITH PERIPHERAL GLUCOSE INJECTIONS. M.E. RAGOZZINO, M.E. PARKER AND P.E. GOLD. Dept. Psychol., U. Virginia, Charlottesville, VA 22903.

ß-endorphin injected into the medial septum impairs spatial memory (Bostock et al., *Behav. Neurosci., 102*, 643). Peripheral glucose injections attenuate amnesias produced by several treatments, including opiate agonists. Here, we determined whether morphine injected into medial septum impairs memory in spontaneous alternation and inhibitory avoidance tasks and whether peripheral glucose administration attenuates the deficits. Rats received morphine sulfate (3 μg in 1 μl) into the medial septum 30 min prior to testing for spontaneous alternation performance. Morphine-treated rats had significantly lower alternation scores than did CSF-injected controls. Glucose (100 mg/kg, IP), administered at the time of morphine injection, blocked the impairment. With treatments as above, rats were subsequently trained in an inhibitory avoidance task and tested for retention 24 hr later. Morphine-treated rats had significantly lower retention scores than did controls and this impairment was reversed by glucose. Thus, morphine injected into medial septum impaired two measures of memory and both deficits were reversed by concomitant peripheral glucose administration. These findings are consistent with the view that circulating glucose levels, directly or indirectly, inhibit opiate functions in the medial septum. (Supported by ONR N0001489-J-1216 and NIA AG 07648).

508.6

PARALLEL EFFECTS OF THE NMDA ANTAGONIST NPC 12626 ON SLEEP AND MEMORY: REVERSAL OF THE MEMORY DEFICIT WITH NON-NMDA AGENTS. D.L. WALKER, W.S. STONE and P.E. GOLD. Neuroscience Program & Dept. Psychology, University of Virginia, Charlottesville VA 22903.

We previously demonstrated that NPC 12626 impairs spontaneous alternation (SA) and inhibitory avoidance performance as well as long-term potentiation. In the present study we characterized this drug's effects on sleep patterns. Rats were injected with NPC 12626 30 min prior to a 3-hr recording session. Sleep stages were defined by EEG, EMG, and activity measures. 10 but not 1 mg/kg significantly decreased total sleep (TS), paradoxical sleep (PS) bout duration and the PS/TS ratio. 10 but not 1 mg/kg also impaired the SA performance of these animals. The parallel effects of this drug on sleep and memory may reflect a more general impairment of arousal mechanisms important to both.

We previously found that glucose, naloxone, and physostigmine attenuate SA deficits produced by a variety of treatments. In this study we similarly attempted to reverse NPC 12626 - induced deficits. Mice were injected with NPC 12626 (35 mg/kg) 50 min prior to SA testing, and glucose (250 mg/kg), physostigmine (0.01 mg/kg), or naloxone (1 mg/kg) 30 min prior to testing. Each drug reversed the effects of NPC 12626 without having any effect when administered alone. Thus, some effects of NMDA antagonists can be ameliorated by non-NMDA treatments. We are currently assessing the effects of these treatments on NPC 12626 - induced sleep deficits. (Supported by AG 07648 and ONR N0001489-J-1216; NPC 12626 generously provided by NOVA Pharmaceuticals).

508.7

GLUCOSE ENHANCEMENT OF MEMORY IN ELDERLY HUMANS: C.A. MANNING, M.W. PARSONS and P.E. GOLD. Dept. Psychology, U. Virginia, Charlottesville VA 22903.

Glucose improves performance on secondary memory tasks in elderly humans. To dissociate glucose effects on storage from acquisition, we examined the effects of posttraining glucose on memory in elderly humans. In addition, a preliminary dose-response curve of glucose effects on memory was carried out.

Subjects aged 60-81 (n=23) heard a narrative passage and were given immediate posttraining or pretraining glucose, or saccharin. Recall, tested 24 hours later, was significantly better in both glucose conditions than in the saccharin condition. Next, adults aged 60-82 (n=10) were given beverages with 10g, 25g or 50g glucose and 50.6 mg saccharin. After ingestion, subjects took memory tests previously enhanced by glucose. The 25g glucose dose significantly improved performance relative to saccharin with a trend for improvement at the 10g dose.

These studies further define the effects of glucose on human memory. The finding that posttraining glucose enhances memory suggests that the treatment acts on memory storage processes. Glucose enhancement of memory outlasts the increase in blood glucose levels, as seen in the 24-hour recall enhancement with both pre- and posttraining glucose. Finally, the selective enhancement at 25g suggests that, as in animal studies, glucose effects on memory are characterized by an inverted-U dose-response curve. (Supported by AG 07648 and ONR N0001489).

508.8

THE MEMORY RETRIEVAL EFFECTS OF IDAZOXAN DEPEND ON THE DEGREE OF FORGETTING. M. Bunsey, J. Horne* and B.J. Strupp. Division of Nutritional Sciences and Department of Psychology, Cornell University, Ithaca, NY 14853.

We previously observed that the effect of AVP4-9 on memory retrieval is critically dependent on the accessibility of the memory at the time of injection. A single dose of the peptide enhanced memory at long retention intervals when memory was weak in control rats and impaired memory at short retention intervals, when memory was strong in controls. This pattern of results suggests that the injected peptide was interacting with endogenous changes corresponding to the strength of the memory. Using the same protocol and memory test (social learning paradigm), the present study was designed to determine if this same pattern of results would be produced by pretest administration of a drug that increases norepinephrine (NE) release, the α_2 antagonist idazoxan (IDA). This hypothesis was based on the evidence that: (1) the mnemonic effects of VP are mediated, in part, by catecholamines, (2) changes in central NE metabolism at retrieval have been shown to correlate to recall, and (3) retrieval has been shown to be facilitated by IDA. Pretest IDA (2 mg/kg) produced the same pattern of results as previously observed with AVP4-9, suggesting that endogenous NE activity at the time of recall varies with accessibility of the memory.

508.9

THE EFFECTS OF ESSENTIAL ELEMENTS ON LEARNING AND MEMORY IN MICE. Q.S. Deng and Y. Yin*. Dept. of Pharmacology, Nanjing Tie Dao Medical College, Nanjing, China 210009

Previous studies showed that essential elements are universally required for survival (Frieden E. Biochemistry of the essential ultratrace elements. Plenum Press, 1984). We have examined the hypotheses that learning and memory are closely related to some essential elements. Studies were conducted by testing of mice in an Y-maze. Male mice (25-32 g) were divided into 10 groups (N=15 in each group). Animals were fed with standard food but different water containing various essential elements. The Choice accuracy which was the number of correct choice in 10 trials was determined after 3 weeks. Those elements: Na (5.4×10^{-2} M), Mg (1×10^{-1} M), Cu (1.6×10^{-4} M), and Fe (2.7×10^{-5} M), increased the choice accuracy significantly. However, others (Cl, Mn, Zn, Al, K and tap water) did not affect choice accuracy. The results of the present study suggest that some essential elements (Na, Mg, Cu and Fe) have positive effects on leaning and memory in mice. (supported by Natural Science Foundation of China #0389001).

508.10

SCOPOLAMINE INTERACTIONS WITH SELECTIVE D1 AND D2 AGONISTS AND PERFORMANCE IN THE RADIAL-ARM MAZE. E. D. Levin and J. E. Rose. Department of Psychiatry, Duke University Medical Center, Durham, NC 27710.

There is evidence for a reciprocal interaction of dopaminergic (DA) mechanisms with muscarinic systems in terms of cognitive function. The adverse effect of scopolamine in the radial-arm maze (RAM) choice accuracy can be counteracted by DA blockade. This study examined the interactions of D1 and D2 agonists with scopolamine effects on RAM choice accuracy. Eleven adult female Sprague-Dawley strain rats were injected (SQ) 20 minutes before testing with normal saline, scopolamine (0.15 mg/kg), the D1 agonist SKF 38393 (2 and 4 mg/kg), the D2 agonist quinpirole (0.04 and 0.08 mg/kg) and each of the D1 and D2 agonist doses with scopolamine. None of the doses of the D1 and D2 agonists by themselves showed signs of reducing choice accuracy. The D2 but not the D1 agonist slowed the rate of responding. Scopolamine by itself caused both a decline in choice accuracy and an increase in choice latency. These effects were not counteracted by either the D1 or D2 agonist. In fact, the D2 agonist showed greater than additive effects in slowing the rate of arm entries. There was an indication that the D2 agonist in combination with scopolamine caused a further decline in choice accuracy. This effect of exacerbating the effect of scopolamine-induced choice accuracy impairment contrasts with the D2 agonist reversal of the RAM choice accuracy impairment caused by the nicotinic antagonist mecamylamine. (Research supported by a grant from the United Way)

508.11

AMPHETAMINE EFFECTS ON JUMP-UP AVOIDANCE AND CAUDATE DOPAMINE METABOLISM AS MEASURED BY HPLC IN VIVO DIALYSIS P.H. Janak, S. Shibanoki*, K. Ishikawa* and J.L. Martinez, Jr. Dept. of Psychology, Univ. of California, Berkeley CA & *Dept. of Pharmacology, School of Medicine, Nihon Univ., Tokyo, JAPAN

Each day for 2 days rats received 1 avoidance trial using a 290uA footshock delivered to the floor of an automated shelf-jump box 10 s after placement in the chamber. Rats were allowed to escape shock delivery by jumping onto a raised shelf. Immediately after each training trial, animals received AMPH or saline, IP. Retention was tested by training to a criterion 24 hr later. AMPH (1 mg/kg) facilitated acquisition of the jump-up response ($F(1,7)=10.53$ $p<.02$). In separate animals implanted with cannulas, caudate dopamine (DA), DOPAC and HVA levels were measured following IP injection of AMPH. Dialysate samples were collected at 20 min intervals for 3 hr after injection and assayed with HPLC-ECD. A 1 mg/kg dose increased striatal DA levels 20-40 min after injection to 518+71.85% of baseline. DOPAC and HVA decreased 40-80 min postinjection by 39+2.67% and 66+3.83%, respectively. Supported by DA06192 DA04795 and DA05375.

508.12

D-AMPHETAMINE INCREASES STRENGTH BUT DOES NOT DECREASE LATENCY OF SPINAL FIXATION IN RATS. M.J. Bartelt, A.G. Hutchins*, L.E. Kerns*, J.M. Guidetti*, and M.M. Patterson Department of Psychology and College of Osteopathic Medicine, Ohio Univ. Athens Ohio, 45701

It has been demonstrated that lasting hindlimb flexion (spinal fixation) may be induced by external stimulation to the upper right hindlimb in spinalized rats (Steinmetz *et. al., J.C.P.P.,* 95:4, 548-555, 1981). Temporal parameters of spinal fixation were examined by Steinmetz, *et. al.,* (*J.C.P.P.,* 96:2, 325-327, 1982). In 1989, Bartelt *et.al.* (*Neurosci. Abst.*,15:467, 1989) found d-amphetamine to significantly increase the amount of hindlimb asymmetry. The present study was done to establish the temporal parameters of spinal fixation under d-amphetamine.

In the present study, 70 rats were anesthetized with Nembutal (50 mg/kg i.p.) and randomly assigned to one of seven groups of 10, 20, 25, 30, 40,45, and 50 minutes stimulation time. Animals were administered d-amphetamine (2mg/kg i.p.) 15 minutes prior to stimulation and then spinalized at T7. Immediately following upper hindlimb stimulation (3-4mA, 100-pps, 7msec, repetitive dc pulses), fixation was measured as the amount of weight needed to remove hindlimb asymmetry.

Results show a generalized increase in the asymmetry for all time groups when compared to results of Steinmetz, *et.al.,* 1982. with a noted plateau of asymmetry occurring between stimulation times of 30 and 45 minutes. The asymmetry strengthening effect of the d-amphetamine increased with increases in time of stimulation. This work supports earlier work by Bartelt *et.al.* ,1989, which suggested catacholamine involvement in the fixation. Further work will include a dose-response evaluation of d-amphetamine.

508.13

FACILITATION OF LEARNING AND MEMORY FOLLOWING ADMINISTRATION OF THE 5-HT3 RECEPTOR ANTAGONISTS ZACOPRIDE AND DAZOPRIDE IN MICE. H. J. Altman[1] and R. F. Berman[2], [1]Department of Psychiatry, Wayne State University School of Medicine, Detroit, MI 48207, [2]Department of Psychology, Wayne State University, Detroit, MI 48202.

Recent evidence points to the existence of multiple serotonin (5-HT) receptor subtypes within the CNS. Presently, three major subtypes of 5-HT receptors have been identified - designated 5-HT1, 5-HT2 and 5-HT3. Over the past several years this laboratory has been systematically investigating the role(s) each of these purported 5-HT receptor subtypes may play in the mediation of the processes underlying learning and memory. The purpose of the present series of studies was to examine the effects of acute pre-training and/or pretesting blockade of 5-HT3 receptors on learning and memory in mice trained in a shock-motivated Y-maze visual discrimination task and a one-trial inhibitory avoid ance task. Significant improvements in learning and/or memory were seen in both tasks, thus providing evidence for a possible role for 5-HT3 receptors in the mediation of the processes underlying learning and memory in mice trained in these two tasks.

508.14

EFFECTS OF COMBINED CHOLINESTERASE INHIBITION AND SEROTONERGIC RECEPTOR BLOCKADE ON AGE-RELATED INHIBITORY-AVOIDANCE RETENTION DEFICITS IN RATS. H.J. Normile and H.J. Altman. Dept. of Psychiatry, Wayne State Univ. Sch. of Med., Detroit MI 48207

We recently reported that post-train administration of serotonergic receptor antagonists attenuated avoidance retention deficits normally exhibited by aged rats. In the present study, we determined whether subeffective doses of one such antagonist, ketanserin, would augment the facilitative effects produced by the cholinesterase inhibitor, physostigmine, on memory in aged rats using the same task. The drugs were injected ip alone, or in combination, immediately following training. Retention testing occurred 24 hrs following training. A dose-dependent enhancement of memory was demonstrated as a result of the three treatment conditions (i.e., ketanserin, physostigmine, ketanserin + physostigmine). The facilitation of memory produced by the combined treatment was observed at doses well below those required to produce a similar effect when each drug was administered alone. The results provide additional evidence for an interaction between the cholinergic and serotonergic neurotransmitter systems in learning and/or memory, and may have important implications in the treatment of geriatric-related cognitive disorders. (Supported by NIA grant #AG07069 and ADRDA grant #RG87087).

508.15

p-CHLOROAMPHETAMINE'S MEMORY IMPAIRING EFFECTS IN RATS ARE DUE TO SEROTONERGIC RELEASE NOT DEPLETION. A.C. Santucci, R. Gluck*, J. Bangston*, M. Gerard* and V. Haroutunian. Bronx VAMC & Mt. Sinai School of Medicine, New York, NY 10468.

The present investigation examined whether the memory impairing effects of p-chloroamphetamine (PCA) are due to the drug's serotonergic release or depletion consequences. In Expt. 1, rats received PCA or saline at various intervals before passive avoidance training and were then tested 24 hr later. Results indicated that PCA (2.5 mg/kg) impaired retention relative to saline-injected controls only when the drug was given shortly (15 min - 2 hr) before training (ps < .05). In Expt. 2, animals were prepared with 5,7 DHT (30 μg/2 μl, DMI pretreatment) or sham lesions of the nucleus basalis of Meynert (nbM). Two weeks postoperatively subjects were administered 2.5 mg/kg PCA or saline 30 min prior to passive avoidance training and were then tested 72 hr later. Retention deficits were observed only in rats treated with PCA irrespective of the animal's lesion status (ps < .05). However, both the PCA treatment and the 5,7 DHT lesion procedure produced stern serotonergic depletions in the frontal cortex (44% - 66%) and in the nbM (50% - 85%) (ps < .05). It is concluded that the memory deficits following PCA administration are due to the drug's serotonergic release effects at sites other than the frontal cortex or the nbM.

509.1

RIPLEY'S "BELIEVE IT OR NOT!": AN HISTORICAL REPRESENTATION OF NEUROSCIENCE FACTS AND ARTEFACTS IN THE POPULAR PRESS. R.A. Johnson. Behavioral Neuroscience Program, Department of Psychology, UCLA, Los Angeles, CA 90024.

In 1918, New York sports cartoonist Robert Ripley published the first of what would prove to be untold thousands of facts and factoids, under the rubric "Believe It or Not!" (BION). In his worldwide pursuit of truth that was stranger, or at least more interesting, than fiction, Ripley could not help but encounter and advertise some of Neuroscience's more intriguing anecdotes and artefacts. He and his successors presented these in over 300 newspapers in 17 languages, to a circulation reaching at one point 80 million, in the same stylized black and white illustrations and exclamation-pointed statements as his other claims.

Ripley's biographers have noted that "his was a mind uncluttered by culture", and that he had "the curiosity of the unlearned". He was interested in the fascinating, the curious, and the bizarre, with often-reprinted neuroscience oddities such as Phineas Gage and trephined skulls falling among the steady stream of topics teasing the public's seemingly insatiable appetite for anecdotes and minutiae. The popular impact of BION is undeniable; BION was a national institution, a forerunner of modern tabloids in the appreciation of the *facts* of science without consideration of the scientific *process*, but with a reputation for scrupulous documentation of its claims.

This poster is the beginning of a project examining the popular representation and perception of Neuroscience. Neuroscience-themed BION panels will be presented, compared with their original sources, and examined with respect to salience of interpretation and accuracy of content. Attendees of the Annual Meeting will be invited to recollect and discuss encounters with BION and other popular sources, especially as the popular accounts sparked personal interest in the neurosciences.

509.2

THE HERRICKS AS NEUROSCIENTISTS. II. CHARLES JUDSON HERRICK 1868-1960. L. H. Marshall and H. W. Magoun, Brain Research Institute, UCLA, Los Angeles, CA 90024.

The youngest of four brothers who were reared on a frontier midwestern farm that provided the major subsistence for the Baptist minister's family, Charles Judson Herrick assimilated nature, a Christian attitude, and intellectual expectations. He followed his ten-years-older brother, Clarence, to Denison University and the University of Cincinnati and returned to Denison to be associated with him in a new graduate biology program. Charles Judson received his Ph.D. from Columbia University in 1900. After he returned to Denison, he continued a commitment made in 1894 that was of greatest importance to the progress of neuroscience: managing editorship of the Journal of Comparative Neurology, when Clarence became desperately ill. For 30 years he was the journal, setting type when necessary. Called to the University of Chicago in 1907, Herrick began teaching medical students. He also organized a graduate course that attracted students and faculty from many disciplines. Herrick's writings became prominent after he retired in 1934, the happiest and most fruitful years of his career. His most important publication was The Brain of the Tiger Salamander (1948). Not content solely with descriptive research in elegant detail, Herrick explained and amplified his ideas in psychobiology, to "... find out what these animals do with the organs they have and what they do it for.... "

509.3

THE HERRICKS AS NEUROSCIENTISTS I. CLARENCE LUTHER HERRICK 1858-1904. H. W. Magoun and L. H. Marshall, Brain Research Institute, UCLA, Los Angeles, CA 90024.

Clarence Luther Herrick was born near the thriving frontier village of Minneapolis, Minnesota. His interest in nature and intellectual precocity led him, with three adolescent friends, to form the Young Naturalists Society, with serious papers delivered at regular meetings throughout their high school period. By graduation from the University of Minnesota, he had published five articles on natural history topics in scientific journals. During a year's postgraduate study in Leipzig, Herrick translated Rudolf Hermann Lotze's booklet, Outlines of Psychology (1881). As professor of natural history at Denison College Granville, Ohio, he formalized in his teaching and writing his concepts of psychobiology, and realized the productiveness of the ontogenous and phylogenous approach to the brain-mind problem. Three years in Cincinnati saw the peak of his research career and the launching of the Journal of Comparative Neurology in 1891. An aborted appointment at the new University of Chicago held great hopes that Herrick would be able to form a department integrating interdisciplinary studies in neuroscience. The University president, William Rainey Harper, reneged on several promises, however, and Herrick felt he could only resign. When tuberculosis struck, he went to New Mexico and became briefly the first president of its university. He died there at 46 years of age.

509.4

PRESERVED PRIMING OF NOVEL STIMULI IN AMNESIA: EVIDENCE FOR MULTIPLE MEMORY SYSTEMS. M.E. Smith (1,2) & M. Oscar-Berman (2). 1. Psychology, Texas A&M University, College Station, TX, 77843; 2. Boston Univ. School of Medicine.

Repetition priming of items with pre-existing codes in memory (e.g. words) might be mediated either by activation of those codes, or by new learning. In contrast, priming of novel stimuli (e.g. pseudowords) probably requires new learning. Consistent with the activation view, studies of perceptual identification (Cermak et al., 1985, Neuropsychologia) and stem-completion (Diamond & Rozin, 1984, J. Abnml. Psychol.) have found spared priming of words in amnesia, but impaired priming of pseudwords. In the present study we measured repetition priming of words and pseudowords in a lexical decision task, comparing data from 8 Korsakoff amnesic patients and matched control subjects. We also found normal priming of words and no priming of pseudowords in amnesia, but only for RT data. The amnesics patients also demonstrated an enhanced tendency to misclassify repeated pseudowords as words. This indicates a preserved ability to acquire novel information in amnesia, and is consistent with multiple memory-system theories (e.g. Tulving & Schacter, 1990, Science).

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509.5

HUNTINGTON'S PATIENTS LEARN MOTOR ASSOCIATIONS, BUT NOT MOTOR SEQUENCES. D. B. Willingham and W. Koroshetz. Dept. of Psychology and Dept. of Neurology, Harvard U. and Mass. Gen. Hosp., Cambridge, MA 02138.

The striatum is implicated in motor learning (Heindel, et al, 1989). The present work tested motor learning in Huntington's Disease (HD) to test whether the striatum subserves learning all motor skills or only a subset.

In Experiment 1 one of four lights appeared, and subjects pushed a button below the light. Subjects were not told that the sequence of lights repeated. Control subjects were unaware of the sequence, yet showed sequence-specific learning by response times that decreased, then increased when switched to random lights. HD patients did not show sequence-specific learning.

Experiment 2 eliminated the repeating sequence and made the stimulus-response mapping incompatible. Under these conditions HD patients learned the task normally.

Striatal damage affects tasks requiring association of visual cues and motor responses, but not tasks based on sequences of responses. This dissociation suggests independent subsystems of motor learning. We hypothesize that the striatum sets up rapid, open-loop sequences of movements, possibly through connections with supplementary motor cortex. Somatosensation, not vision or kinesthesia, guides these movements. The association of visual cues and motor responses operates independently of the striatum, possibly in premotor cortex (Passingham, 1985).

509.6

VISUAL RECOGNITION MEMORY IN 8- AND 12-MONTH OLD HUMAN INFANTS. M.H. Kates and M. Moscovitch, Dept. of Psychol. U. of Toronto, Erindale Campus, Mississauga, Ont., Canada L5L 1C6

A direct reinforcement version of a delayed non-matching-to-sample task (DNMS) was used to assess visual recognition memory in 48 8- and 80 12-month old human infants. The task involved allowing infants to become familiar with a toy, and then recording whether an infant reached for the novel or familiar toy in a pair. Each session included 12 or 18 trials with no delay and either 40, 80, or 200 sec. between familiarization and test. In addition, the delay period was either unfilled or filled with interference caused by presenting other toys for later testing. When the delay period was unfilled, both groups successfully chose the novel toy at the 80 sec. delay interval. However, when interference was interpolated during the delay, the 8-month olds performed at chance at the 80 and 200 sec. delays, whereas the 12-month olds performed well above chance, although some forgetting was noted. The performance of infant humans on DNMS is compared to that of normal infant monkeys and adult monkeys with hippocampal lesions. It is suggested that by 12 months of age, but not by 8 months, the human hippocampus is sufficiently developed to mediate performance across long delay intervals filled with interference.

509.7

FURTHER CHARACTERIZATION OF THE VISUAL PROCESSING DEFICIT IN NEPHROPATHIC CYSTINOSIS. S.N.Nichols*,A.O.Ballantyne*, B.L.Hodge* and D.A.Trauner. Dept. of Neurosciences, Univ. of California, La Jolla, CA 92103

Previous studies have demonstrated an isolated deficit in visual processing in children with infantile nephropathic cystinosis, a hereditary lysosomal storage disorder. Children with this disorder appear to have difficulty both with visual memory and with manipulation of mental images. In order to further characterize the problem, 11 children with cystinosis and 11 matched controls were studied using a visual memory task, the Benton Visual Retention Test (VRT).

Cystinotic subjects made significantly more errors involving reproduction of internal details of figures on the VRT than did controls (1.9±1.6 vs. 0.9±1.1, p=.02). The 2 groups did not differ significantly in their tendency to distort the overall shape of the figures. Preliminary analyses of 2 other tasks, Gollin Incomplete Figures and Closure Speed, suggest that cystinotics may perform more poorly on tests involving identification of incomplete figures, a skill requiring integration of detail.

These data suggest that children with cystinosis may be impaired in their ability to maintain details of an image in memory or to form an accurate mental image when incomplete visual information is presented.

509.8

PROCESSING OF SPATIAL RELATIONS WITHIN AND BETWEEN THE DISCONNECTED CEREBRAL HEMISPHERES. J. Sergent. Montreal Neurological Institute, McGill University, Montreal, Canada.

This study examined 3 issues related to the processing of spatial relations by commissurotomized subjects. (1) The respective competence of the disconnected hemispheres at performing judgments of relative position and distance between two objects was tested. The results showed that the two hemispheres were equally competent at representing and operating on these spatial-relation representations. (2) The capacity of the disconnected hemispheres to operate conjointly was then examined. The two disconnected hemispheres were simultaneously stimulated with the same information and had to produce a single response based on this information. Compared to unilateral stimulations, bilateral stimulations resulted in enhanced response accuracy and, depending on the type of decision, in patterns of response latency and accuracy different from the patterns of either unilateral condition. These results suggest that the two disconnected hemispheres can operate simultaneously and are able to join the outcomes of their respective operations before the production of a single response. (3) Interhemispheric communication of visuospatial information was studied. Unlike pattern information that is typically confined to the hemisphere that receives it in the commissurotomized brain, visuospatial information could be subjected to interhemispheric comparison as a function of its categorical and metric properties, although the patients had only implicit knowledge of part of the transferred information.

509.9

NORMAL ORGANIZATION OF CATEGORY KNOWLEDGE IN ALZHEIMER'S DISEASE. A. Cronin-Golomb, A. Kokodis*, S. Corkin, J.H. Growdon. Dept. of Brain and Cognitive Sciences, MIT, Cambridge MA 02139, Dept. of Psychology, Boston University, Boston MA 02215, and Dept. of Neurology, Massachusetts General Hospital, Boston MA 02114.

Deficits in category knowledge in Alzheimer's disease (AD) may be attributable to a disruption in underlying organization, or to impaired retrieval of correctly organized information. We addressed these alternative possibilities with 3 tasks: (1) category decision (Is __ a member of [category]?), (2) ranking of category exemplars by typicality (e.g., airplane>tractor>sled as 'typical' types of vehicles), and (3) fluency (name as many members of [category] as possible in 1 minute). Comparisons of 18 patients with AD, who varied in dementia severity, and 14 age-matched control subjects (CS) revealed highly similar patterns of performance. Certain of the 10 categories proved more difficult than others in all 3 tasks. The correlation between AD and CS performance was 0.83 for Task 1, 0.88 for Task 2, and 0.98 for Task 3. The pattern similarity existed despite other group differences, e.g., slower reaction times for AD than CS in Task 1, and production of fewer items by AD than CS for Task 3. The results suggest that, although retrieval of category information is impaired in AD, the underlying organization of category knowledge is normal.

509.10

COGNITIVE ABILITIES IN LESBIANS. C.M. McCormick and S.F. Witelson, Departments of Psychology and Psychiatry, McMaster University, Hamilton, Canada.

We previously reported an increased prevalence of non right-hand preference in homosexual women and interpreted the result as evidence that atypical cerebral asymmetry is a factor in homosexuality (McCormick et al., 1987, 1990). In the present study, we compared homosexual females to heterosexual females and males (n = 31 per group), matched for hand preference, on cognitive tests that typically reveal differences between males and females (spatial ability, fluency). Previous results with homosexual males (Witelson & McCormick, 1989) indicated lower spatial ability and higher fluency in homosexual males than in heterosexual males. In contrast, no cognitive differences were found overall between homosexual and heterosexual females. However, among non right-handed subjects, homosexual females scored lower on cognitive tests than did heterosexual females. These results are examined with reference to neuropsychological studies of women exposed to atypical levels of prenatal hormones.

509.11

A COGNITIVE BEHAVIOURAL DEFICIT ASSOCIATED WITH HIV-SEROPOSITIVITY. B.D. Fantie§†, A.F. Mirsky†, and R.T. Bowes*, §Neuropsychology Laboratory, Department of Psychology, The American University, Washington, D.C. 20016-8062, and †Laboratory of Psychology and Psychopathology, National Institute of Mental Health - Intramural Research Program, Bethesda, MD 20892.

Although there is clear evidence that the brain is a primary site of human immunodeficiency virus (HIV) infection, controversy exists concerning whether the early presence of HIV is associated with any concomitant cognitive or behavioural dysfunction detectable through standard neuropsychological assessment. In this preliminary report we present three cases of such a deficit, manifesting as an alteration in the ability to perform the Wisconsin Card Sorting Task (WCST), in a population of young adults likely to be particularly vulnerable to central nervous system compromise. In contrast to 14 seronegative controls, 3 HIV+ patients showed a decline in performance on certain components of the LPP-Attention Battery during a 3-6 month follow-up session. Specifically, they completed fewer categories and made more 'Failure to Maintain Set' errors on the WCST in comparison to both controls and their own initial performance. Their scores on other tasks either remained stable or improved across sessions, indicating that the decline on the WCST was unlikely to be the result of some general motivational effect. There is great practical importance in the early detection of cognitive/behavioural dysfunction associated with HIV seropositivity.

509.12

LAUGHTER: SOCIAL CONTEXT, STRUCTURE, AND CONTAGION. R. R. Provine. Dept. of Psychology, Univ. of Maryland Baltimore County, Baltimore, MD 21228.

Laughter is an ubiquitous, species-typical human signal and motor act. Laughter is almost exclusively social, even more so than smiling and talking; it occurs 30 times more frequently in social than in solitary settings. Laughter is characterized by stereotyped laugh note duration ("ha") and inter-note-interval ("ha-ha-ha...."). This stereotypy contributes to the acoustic signature of laughter and is the stimulus for laugh-induced ("contagious") laughter. Although the contagiousness of laughter is widely known and has even spawned the technology of the "laugh track" on broadcast comedy shows, the ramifications of this extraordinary behavior for human brain function have not been appreciated. The potent contagious laughter effect suggests that humans have evolved a neurological acoustic feature detector that evokes laughter when triggered by a stimulus having the unique stimulus properties of laughter. The stereotypy of laughter as an acoustic stimulus and motor act and the presence of the contagious laughing effect suggests that modular mechanisms are involved in both laugh production and reception. These data provide insights into the curious behavior of laughter, the evolution of language, and a variety of issues in cognitive neuroscience.

509.13

DOSE EQUIVALENCY: A HUMAN BEHAVIORAL BIOASSAY TECHNIQUE. R. S. Kennedy, J. E. Fowlkes*, W. P. Dunlap,* and Janet J. Turnage*. Essex Corporation, Orlando, FL 32803.

It is a well-known fact that human performance is impaired by drugs, toxic elements, environmental stress, illness, emotional strain, and nutritional deficits. Scientific study of how these factors influence performance in the workplace is hindered by the lack of suitable metrics. In a series of experiments, various treatments (e.g., halon, hyoscine, hypoxia, alcohol) and holistic measures of mental acuity (WAIS, ASVAB, Wonderlic, ACT) were given to various groups of subjects. An Automated Performance Test System (APTS) battery of repeated-measures cognitive, perceptual, information-processing, and motor tests was also administered in standardized format in all the studies. Using APTS scores as predictors, regression equations were calculated to determine performance impairment as a function of alcohol dosage (the criterion) in one carefully graded experiment. Performance decrement was correlated with blood alcohol level (r = .88) using a composite APTS score. Regression equations were then calculated for these same tests using the holistic intelligence measures. Then the dozen sensitivity studies, using various drugs, were translated into the effects that those agents had on mental activity using as a transform the performance test battery regression equations. The ability to use such a dose equivalency method to conduct human performance bioassays is discussed as it relates to neuroscience research and for practical workplace decisions.

509.14

DECOUPLING OF PET MEASURED LEFT CAUDATE AND CORTICAL METABOLISM IN ADULT DYSLEXIA. P. Tallal, F. Wood*, M. Buchsbaum*, L. Flowers*, I. Brown and W. Katz*. Center for Molecular and Behavioral Neuroscience, Rutgers Univ., Newark, N.J. 07102.

Positron Emission Tomography (PET) was used to investigate neural mechanisms underlying speech processing disorders in reading impaired (dyslexic) subjects. Ten adult dyslexics, as defined by childhood reading and IQ scores, and ten controls were compared on glucose utilization values obtained from PET reflecting brain activation during a continuous auditory syllable detection and discrimination task. In controls, the glucose utilization values in either caudate were highly correlated with values in all cortical regions, especially those in frontal and temporal areas. In dyslexics, however, correlations were significantly attenuated, and most correlations between left caudate and other cortical regions were near zero. The differences between the correlations in the dyslexics and in the controls were significant at p less than .05.

The results are interpreted in light of our previous report from an MRI study of smaller caudate volumes in language impaired children (Jernigan, T., Tallal, P., and Hesselink, J., Am. Acad. of Neurol., 1989), and in light of theoretical implications for the role of caudate activity in language processing.

509.15

ELECTROCORTICOGRAM COHERENCE AND CORRELATION OF AMPLITUDE MODULATION BETWEEN ELECTRODES BOTH DECLINE IN MILLIMETERS IN HUMAN AS WELL AS IN RABBIT BRAINS. T.H.Bullock, V.J. Iragui* and J.F. Alksne*, Dept. of Neurosciences, U.C.S.D., La Jolla, CA 92093.

Coherence is not typically widespread over the cortex; it may have a significant microstructure. Metal disc electrodes 5-10 mm apart, embedded in plastic strips, inserted under the dura over frontal, parietal and temporal lobes of epilepsy patients recorded electrocorticogram (ECoG) during sleep, wakefulness and seizures. We computed coherence (Coh) for frequencies (F) 1-80 Hz between all 120 pairs among 16 electrodes and plotted Coh vs distance (D) between electrodes, pooling lobes but not states or patients. Averaging over many sec, Coh declines monotonically with increasing D, for all Fs. Coh vs F shows no consistent pattern; differences in Coh with F are commonly slight. Though widely different among subjects and electrode sites, a roughly median value of 5-15 mm for Coh=0.5 in humans compares with 2.5-5 mm reported for rabbits (T.H. Bullock & M.C. McClune EEG Clin. Neurophysiol. 73:479-498, 1989). This is probably the best measure of the degree of synchronization in domains of cortex. Between slow-wave sleep and alert states differences in these values are slight. Another dynamic feature, quite distinct from Coh, is the correlation between electrode loci of the amplitude modulation (AM) of narrow ECoG bands. For 6 bands, 1-4, 4-8, 8-13, 13-19, 19-30, 30-45 Hz, we computed AM envelopes, low-passed these to 4 Hz, correlated (AMCor) each pair of electrodes, and plotted this value vs D. AMCor declines with D; at 10 mm mean values are usually 0.4-0.6 for all Fs, slightly more for the lowest; at 20 mm <0.1-0.4; at 30 mm some correlations are just significantly negative. It remains to learn whether these two aspects of cooperativity covary with state or region.

509.16

CURRENT-DENSITY IMAGING AS A METHOD OF VISUALIZING NEURONAL ACTIVITY OF THE BRAIN. Y. C. Okada, J.-C. Huang*. VA/LANL/UNM Center for Magnetoencephalography, VA Medical Center and Dept. Neurology, Univ. New Mexico Sch. of Med., Albuquerque, NM 87108

In magnetoencephalography (MEG) the neuronal generator giving rise to the magnetic field outside the head is commonly represented by a current dipole. In contrast we have investigated the application of MEG as a method for obtaining an image of current densities as a functional map of neuronal activity in the brain. Here we describe a hierarchical deconvolution algorithm for current imaging. As in the linear estimation procedure this method starts with the solution of the linear matrix equation of the form: B=AJ, where B is the vector containing the component of the magnetic field data normal to a field plane, J is a vector of current densities lying tangentially on the source plane and A is the lead-field matrix. In the initial step, the source plane is divided into coarse grids. The solution for J is found from the generalized inverse of A equal to $(A'A)^{-1}A'$, where A' is the transpose of A. The magnitudes of J determines the grid pattern of the source plane to be used in the next step. Those grids containing large magnitudes are subdivided, whereas the rest is left undivided. Using this new grid pattern, the linear equation is solved again. The procedure is repeated iteratively until the minimum grid size becomes less than the spatial resolution of this method determined by the distance between the field and source planes. Simulations showed that this method requires 5-10 times less number of free parameters and faster than the straight-forward single-step, linear estimation technique. The solution is more stable with the former method in the presence of noise in the B field.

Supported by NINDS grant R01-NS21149 and Dept. Veterans Affairs.

509.17

ANATOMICAL CONSTRAINTS FOR NEUROMAGNETIC SOURCE MODELS. **C.C.Wood, J.S. George, P.S. Lewis*, D.M. Ranken*, and L. Heller***. Neuromagnetism Lab., MS M715, Los Alamos National Lab., Los Alamos NM 87545.

The goal of neuromagnetic recording techniques is high resolution mapping of human brain activity. This goal requires the solution of an ill-posed inverse problem; namely, the determination of the number, location, spatial configuration, strength, and timecourse of the neuronal currents that give rise to the magnetic field distribution at the head surface. To help resolve ambiguities in the source modeling process, we are using a system for anatomical segmentation and volumetric reconstruction based on MR images (George et al., *Soc. Neurosci. Abs.*, 1989, 15:730) to investigate alternative strategies for constraining the locations of allowable sources. One strategy uses proximity to the local cortical surface to generate an error term that is incorporated into a standard least-squares minimization algorithm. Another strategy makes use of the fact that surface magnetic fields are a nonlinear function of location but a linear function of source orientation and current strength. Field distributions may therefore be expressed as linear combinations over a basis matrix including field amplitudes at each sensor location for unit current vectors in x, y, and z dimensions. A constrained basis matrix formulation can be combined with pseudo-inverse procedures such as the minimum norm lead field expansion to increase the anatomical realism of mathematical reconstruction procedures.

509.18

MULTI-SOURCE MODELING OF NEUROMAGNETIC DATA: SIMULATION AND EMPIRICAL STUDIES. **S. Supek* and C. J. Aine**. Physics and Life Sciences Divisions, MS M715, Los Alamos National Laboratory, Los Alamos, NM, 87545

A critical step in attempting to model the surface magnetic fields generated by the human brain is determining the number of sources (i.e., the "order" of the model). To address this question we conducted extensive simulation studies using point current dipole sources in a homogeneous spherical conductive medium in which the number, spatial configuration and strength of sources and amount of noise added were systematically varied.

Three approaches to the identification of model order from the simulated instantaneous magnetic field distributions were evaluated: (1) visual inspection of isofield contour maps; (2) comparison of percent of variance accounted for by single- versus multi-source models; and (3) examination of the reduced chi-square values (chi-square/degrees-of-freedom) for alternative models. When the true noise level is known, the reduced chi-square approach provides the most sensitive and valid means for determining model order. However this method is sensitive to both the accuracy of noise estimates (for empirical data) and the magnitude of the noise. For example, when the noise level is high, a chi-square test may indicate that a single source configuration could have generated a field pattern actually produced by multiple dipoles.

This approach has been applied to empirical neuromagnetic data elicited by the simultaneous and separate presentation of visual stimuli.

510.1

EFFECTS OF FOREBRAIN LESIONS ON DRINKING AND SALT APPETITE AFTER DOCA OR YOHIMBINE. D.A. Fitts. Dept. of Psychology, Univ. of Washington, Seattle, WA 98195.

Lesions of the ventral part of the ventral median preoptic nucleus (VVMnPO) enhanced daily salt appetite induced by sc injections of 0.5 to 5 mg/kg/day deoxycorticosterone acetate (DOCA), but had no effect on acute salt appetite or water intake after one sc injection of 5 mg/kg yohimbine (yohimbine method: Johnson, et al., Soc. Neur. Abs., 15, 964, 1989.) Lesions of the subfornical organ (SFO) had no effect on daily saline or water intakes during 3 mg/kg/day DOCA, but significantly reduced acute water intake after yohimbine. Pretreatment with a large dose of 100 mg/kg captopril ip did not significantly reduce either the water or saline intakes after yohimbine injections in normal rats, so these behaviors do not appear to depend on peripheral angiotensin synthesis. The findings demonstrate that the enhanced DOCA-induced saline intake in VVMnPO-lesioned rats is specific to lesions of the ventral lamina terminalis. By contrast, the VVMnPO lesion reduces salt appetite after angiotensin-related treatments such as captopril or sodium depletion, and has not been found to affect stimulated water intake (Fitts, et al., Behav. Neurosci., in press). SFO lesions do not affect salt appetite in daily intake tests, but do reduce acute or chronic water intake after treatments such as angiotensin, captopril or yohimbine. Supported by NS22274.

510.2

HEPATIC VAGOTOMY DOES NOT REDUCE THE SATIATING EFFECT OF A NaCl PRELOAD ON DEPLETION-INDUCED NaCl INTAKE. S.P. Frankmann, C.J. O'Connor* and G.P. Smith. Bourne Lab., Dept. of Psychiatry, New York Hospital-Cornell Univ. Med. Coll., White Plains, NY 10065.

The hepatic branch of the vagus nerve has been suggested to be necessary for the normal satiation of depletion-induced NaCl appetite (Tordoff et al., 1986, 1987). If this is true, removal of the hepatic vagus should result in 1) an increase in NaCl intake and 2) less decrease in NaCl intake when given a preload of NaCl. To test this, depletion naive, male, Long-Evans rats were given either sham (S, n=12) or hepatic (H, n=12) vagotomies. Following recovery, all rats were sodium depleted by Lasix (furosemide, 10 mg, sc) and overnight sodium-deficient diet and water. At 18 h later, half of each surgical group received no load (NL) or a load (L) of 7.5 ml of 0.5 M NaCl into the stomach. Ninety min later, the rats were offered 0.3 M NaCl and water in a 1-hr, NaCl-appetite test.

At the end of the first 15 min, the hepatic vagotomized group showed a normal NaCl intake (H/NL = 5.6±1.0 ml; S/NL = 5.9±0.9 ml) and a normal supression of NaCl intake to the preload of NaCl (H/L = 3.3±1.0 ml; vs S/L = 3.3±0.9). The preload produced a significant suppression of NaCl intake ($F(1,20)=7.96$, $p<0.01$), but there was no effect of surgical group ($F(1,20) = 0.29$, $p = 0.60$). At 60 min, there were still no significant differences between the H/L (4.0±1.2 ml) and S/L (3.3±0.9 ml) groups. The H/NL (6.5±0.85 ml) and S/NL (8.9±0.9 ml) groups were not significantly different ($F(1,10) = 3.18$, $p = 0.11$), although the intake from 15-60 min was 46.4% less for the H/NL group.

If the hepatic vagotomized rats had a more rapid emptying of the NaCl preload, this could obscure a satiety deficit. To test this, the rats were sodium depleted again and 90 min after the NaCl preload (1.48 mEq), they were anesthetized and their stomachs were removed. The stomach content volumes were measured and the sodium concentrations were determined by flame photometry. There were no differences in the residual stomach sodium content (mEq) between the two groups (S = 0.117 ± 0.043; H = 0.096 ± 0.042). Thus, under our conditions, the hepatic vagus was not necessary for a normal satiation of NaCl appetite.

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510.3

SODIUM SENSITIVITY AND ORIGINS OF THE PRIMARY POLYDIPSIA OF THE INBRED MICE. K. Koizumi, K. Inenaga*, N. Akamatsu* and H. Yamashita. Depts. of Physiology, SUNY Health Science Center at Brooklyn and University of Occupational and Environmental Health, Kitakyushu, Japan.

The excessive drinking (5-8 times that of normal) of the inbred mice, STR/N, which is not due to lack of vasopressin nor renal dysfunction, was reduced to 35% of control level by low Na^+ diet (10% of regular food). Reduction in water intake began on Day-1 and continued for 9 days after the end of low Na^+ diet which was given for 18 days. In non-polydipsic S/W mice, water intake was reduced to 60% of the control from Day-10 to Day-18 only. Food intake was not much affected in both strains. Since osmolarity and Na^+ concentration of plasma are similar in both strains, the results suggest that the STR/N mice possess an abnormal set-point for Na^+ related to water intake. To test this hypothesis, in brain slice preparations responses of the AV3V-OVLT neurons to changes in osmolarity and Na^+ concentration of the bathing medium were studied using extracellular recordings. No significant differences in their responses were found between the STR/N and S/W. In both groups the neurons were inhibited by hypertonic and excited by hypotonic saline. Replacing Na^+ with mannitol or sucrose did not alter these responses, indicating that no difference existed in sensitivity of the neurons of both strains to Na^+. Our findings suggest that neurons located in the CNS other than in the AV3V may be involved in altered Na^+ sensitivity, or that other mechanisms play a role in the observed attenuation of the polydipsia by low Na^+ diet. (Supported in part by a grant from USPHS, NS-00847).

510.4

SODIUM APPETITE IN RATS AFTER DIETARY SODIUM DEPRIVATION: INHIBITION BY ESTROGEN. E.M. Stricker, E. Thiels and J.G. Verbalis. Depts. of Behavioral Neuroscience and Medicine, University of Pittsburgh, Pittsburgh, PA 15260.

Little sodium appetite is observed when rats are deprived of dietary Na for 4 days, presumably because aldosterone secretion minimizes renal Na losses. When Na deprivation is extended to 8 days, however, a spontaneous sodium appetite results in adult male rats that far exceeds their urinary Na losses during the deprivation period; they ingested 19 ± 2 ml of 0.5 M NaCl solution in 7 hr, which is as much NaCl as rats have ever been reported to drink rapidly. In contrast, female rats drank much less saline after 8 days of Na deprivation (8 ± 1 ml in 7 hr). Because of this sexual dimorphism in sodium appetite, we also studied NaCl intake in gonadectomized rats after 8 days of Na deprivation. Both male and female animals drank comparable amounts of saline as intact males but reverted to the low intakes of intact females when replaced with physiologic amounts of estrogen. These results indicate that a robust sodium appetite can be produced in rats by extended dietary Na deprivation, and that estrogen has a marked inhibitory effect on the induced sodium appetite under these conditions.

510.5

DEPENDENCE OF ANGIOTENSIN-INDUCED NACL INTAKE ON THE ANTEROVENTRAL WALL OF THE THIRD VENTRICLE, "AV3V". L.A. De Luca Jr.*, O. Galaverna*, J. Schulkin*, E. Stellar and A.N. Epstein. Depts. of Biology and Anatomy and Mahoney Inst. Neurol. Sci., Philadelphia, PA 19104.

The structures of the AV3V are rich in angiotensin (ANG) binding sites and may therefore be important for the participation of cerebral ANG in the ANG/aldosterone (ALDO) synergy that underlies increased salt intake in the rat. Sham operated (n=12) and AV3V (n=11, showing deficient dipsogenic response to pulse intracerebroventricular (pICV) ANG II and to s.c. 2 M NaCl) damaged rats were therefore evaluated for the natriorexigenic effects of 1) activation of brain ANG with 100 ng of pICV renin that increased 5 h 3 % NaCl and water intake in shams (8.0 ± 1.9 and 68 ± 13.0 ml, n=6) but not in AV3V (0.3 ± 0.3 and 10.0 ± 4.3 ml, n=6), 2) systemic deoxycorticosterone, DOCA, at 2.5 and 5.0 mg/day, for 3 days. AV3V animals responded to DOCA, but drank less NaCl (376 % vs 580 %) and, unlike shams, did not increase their water intake, and 3) sodium depletion (furosemide and removal of ambient Na for 24 h). Both groups expressed a Na appetite after Na depletion, but shams restored their deficit sooner and drank more water. Tissue in the AV3V and the medial amygdala (MAMY) (Schulkin et al., Behav. Neurosc. 103:1989) may be essential parts of the neural circuit for the ANG/ALDO synergy : AV3V for ANG action and MAMY for the action of ALDO. Supported by MH 43787 & NS 03469.

510.6

THE REVERSAL OF THE SODIUM CHLORIDE AVERSION OF FISCHER-344 RATS BY CHORDA TYMPANI NERVE TRANSECTION. S.I.Sollars* & I.L.Bernstein. Dept of Psychology, University of Washington, Seattle, WA 98195.

Fischer-344 (F-344) rats are atypical in their lack of a preference for any concentration of NaCl solution and their avoidance of NaCl solutions preferred by other rat strains. Whole nerve recordings from the chorda tympani (CT) indicate greater responsiveness of the CT to NaCl stimulation in F-344 rats than in other strains, such as Wistars. Moreover, the exaggerated CT response appears to be associated with greater sensitivity to the sodium channel blocker amiloride. This suggests that when F-344 rats drink NaCl solutions amplified signals from the CT may contribute importantly to their NaCl aversion. The present study examined whether the F-344 rat's NaCl aversion persists after bilateral transection of the CT nerve. In adult male F-344 rats the chorda tympani was sectioned bilaterally (CTX) or was exposed but not sectioned (SHAM). Two bottle preference tests were given to CTX and SHAM rats beginning two weeks after surgery. Concentrations of NaCl (0.6%; 0.8%; 1.0%) maximally preferred by other strains yet avoided by intact F-344 rats were used as the test simuli. At all concentrations tested, CTX animals preferred NaCl solutions to water. This preference differed dramatically from the avoidance of these solutions by SHAM animals. CT cuts in other rat strains have generally failed to significantly affect NaCl preference. That CTX animals preferred NaCl solutions, rather than just failed to avoid them, indicates that they continue to detect the taste of NaCl after CT transection. These findings are consistent with the hypothesis that the F-344 rat's aversion to the taste of NaCl solutions stems from input provided by the CT nerve, particular that component of the CT response which is sensitive to amiloride.

510.7

BILATERAL LESIONS OF THE CENTRAL NUCLEUS OF THE AMYGDALA: EFFECT ON SODIUM INTAKE. Q. Galaverna*, L.A. De Luca Jr*, J. Schulkin*, A. Epstein, University of Pennsylvania; E. E. Coons, New York University.

The central nucleus of the amygdala is the end-station in the ventral forebrain for taste visceral afferents and it is rich in angiotensin-containing terminals. It, therefore, may be an important structure in the neural network that mediates the angiotensin/ aldosterone synergy that underlies increased NaCl intake in the rat. Accordingly, the 3%NaCl and water ingestive behaviors of rats with bilateral ablation of the central nucleus of the amygdala were studied. After uneventful postoperative recovery of food and water intake: 1) daily need-free NaCl intake disappeared both in rats with no prior history of sodium depletion and in those whose intake had been enhanced by several depletions, 2) they did not drink NaCl, but did drink water, in response to SC mineralocorticoid (Mcort), (5mg/day of DOCA), 3) they drank water but did not drink NaCl after activation of brain angiotensin (Ang) with pulse intra-cerebroventricular injection of 100ng of renin, and 4) their NaCl intake after sodium depletion (lasix + removal of ambiant Na+) was blunted. Although its role in salt intake behavior is not yet clear, this work shows that NaCl, but not water intake, depends on intact central nucleus of the amygdala. In their absence, all forms of NaCl intake (need free, Ang-, Mcort-, and depletion-induced) are absent or markedly deficient.
Supported by MH 43787 and NS 03469

510.8

CENTRAL ANGIOTENSIN II (ANG II)-RECEPTOR BLOCKADE REDUCES YOHIMBINE-INDUCED SALT APPETITE. R.L. Thunhorst, K.M. Riggins & A.K. Johnson, Univ. of Iowa, Iowa City, IA 52242.

Subcutaneous (s.c.) injection of the alpha-2 adrenergic antagonist yohimbine (YOH) rapidly produces salt appetite in sodium-replete rats. This effect survives nephrectomy, thus showing independence from the peripheral renin-angiotensin system. We tested for a role of central ANG II receptors by using central ANG II-receptor blockade with sar^1ile^8-ANG II (n=10) or sar^1thr^8-ANG II (n=10) in YOH-treated rats. Rats received lateral ventricular infusions of blocker (15 ug/hr) or vehicle. YOH (3.0 mg/kg) was injected s.c. after 1 hr, and central infusions continued for another 3 hr. Water and 0.3 M NaCl were present throughout. Tests were repeated, alternating the central treatment. Both ANG II-receptor blockers reduced saline, but not water, intakes to s.c. YOH (*p<.05).

	Cum 3 hr intakes after YOH				%
	Water	Saline	Total		Saline
Vehicle	6.1	4.3	13.1	ml	47
Sar^1Ile^8	11.6	1.5*	10.4	ml	15*
Vehicle	8.2	2.6	10.8	ml	22
Sar^1Thr^8	7.6	1.1*	8.7	ml	11*

Thus, central ANG II receptors are importantly involved in salt appetite after YOH treatment.

510.9

AGE-DEPENDENT TASTE PREFERENCES IN RABBITS FOR A SODIUM SACCHARIN SOLUTION. L.J. Dreshfield, L. Fabrigar* and S.D. Berry. Dept. of Psychology, Miami U., Oxford, Ohio 45056.

Preferences for water versus a .22% sodium saccharin solution were obtained for 12 young (4-6 mos., $\bar{X}$=4.7) and 12 old (31-69 mos., $\bar{X}$=53.4) New Zealand white rabbits. Both solutions were offered ad lib in the home cage, and positions were counterbalanced each day for four days. Data were collected an additional six days from many of the subjects to confirm stability of preference. Overall solution intake was not different between groups; older animals consumed an average of 371 ml per day, and younger animals consumed about 487 ml per day ($p < .127^3$). A subpopulation of both young and old animals exhibited a bottle position preference, so data from these animals were excluded from taste preference analysis. Young animals demonstrated a significant preference for water ($\bar{X}$ saccharin consumption 31.5% per day, N=7), while older animals strongly preferred the saccharin solution ($\bar{X}$ intake 78% per day, N=9, $p < .007$). These results suggest that aging increases preference for .22% saccharin solution in rabbits, and may have been responsible for mixed findings in previous taste preference studies. Therefore, we conclude that age is a variable that may affect the incentive value of rewarding stimuli and must be taken into account in studies of learning and motivational processes.

510.10

ANGIOTENSIN 1-7 POTENTIATES ANGIOTENSIN-INDUCED DRINKING. M.J.Sullivan, T.G. Beltz and A.K. Johnson. Ottawa Civic Hospital Research Institute Neuroscience Unit, Ottawa, Ontario, Canada, K1Y 4E9, and Departments of Psychology and Pharmacology and the Cardiovascular Center, Iowa City, Iowa 52242

Aminopeptidase inhibitors potentiate the pressor and dipsogenic effects of angiotensins, probably by inhibiting the degradation of the peptides. In this study we examined the ability of a fragment of angiotensin II (ANG II), angiotensin 1-7 (ANG 1-7), to increase the drinking responses to ANG II. Recent data suggests that ANG 1-7 may effect release of vasopressin (Schiavone et al., 1988) and that ANG 1-7-like immunoreactivity has been localized in rat forebrain (Block et al., 1988). Therefore, we re-examined the dipsogenic effect of the peptides and investigated the effects on fluid and Na balance. Rats were prepared with lateral ventricular cannulae. A dose-response curve to 0, 0.1, 1, and 10 nmol ANG 1-7 was generated. Other groups of rats received icv injections of ANG II with and without a 100-fold higher dose of ANG 1-7. A final group of animals was implanted with osmotic minipumps. Lateral ventricular infusions of artificial cerebrospinal fluid or 2 nmol/ul/hr ANG 1-7 were made and Na and water balance monitored. ANG 1-7 potentiated drinking responses to icv ANG II. As previously reported, ANG 1-7 was not dipsogenic at any of the doses tested nor did it induce salt appetite or affect Na or water balance. We conclude that ANG 1-7 potentiates the actions of ANG II by competing for degradation sites.

510.11

SURGICAL TRANSECTIONS OF POSTERIOR HYPOTHALAMIC CONNECTIONS: EFFECTS ON WATER INTAKE AND RENAL FUNCTION. K.M. Andrews*, M.K. McGowan, G.L. Robertson* & S.P. Grossman. Comm. on Biopsychology, Univ. of Chicago, Chi. IL

Transection of fibers in the posterior hypothalamus produces severe polydipsia which appears to result from disinhibition of a thirst regulatory mechanism. Rats subjected to coronal knife cuts (KC) drank 100-300% more than baseline and sham-lesion (SL) controls. Urinary output increased commensurately. Water intake was reduced similarly by nephrectomy and sham nephrectomy in KC rats, but was still higher than in SL rats. Food deprivation also decreased water intake in KC rats to the level of SL controls, even though food intake remained unaltered by the knife cuts. Water intake and urine output was significantly increased by hypertonic saline i.p. at 0.5, 1 and 8h post-injection. Despite marked differences in input and output, KC and SL rats demonstrated similar basal plasma vasopressin. However, when KC and SL rats were given hypertonic saline i.p., plasma vasopressin rose in SL rats only. Histological analysis confirmed that the knife cuts did not involve the supraoptic nuclei or the neurohypophysis. These preliminary findings suggest that the polydipsia and polyuria in KC rats may be due to a complex mechanism that involves not only decreased secretion and action of vasopressin but an abnormal response to thirst stimuli.

510.12

REGIONAL CEREBRAL BLOOD FLOW FOLLOWING ICV ANGIOTENSIN II IN THE RAT. D.A. Czech[1] and E.A.Stein[2]. Marquette University Dept of Psychology[1] and Medical College of Wisconsin, Dept of Psychiatry[2], Milwaukee, WI 53233.

Angiotensin II (AII) is a potent dipsogen when administered both peripherally & centrally. As its sites of action are not well understood, we attempted to map brain regions affected by ICV AII, and which might be linked to its dipsogenic effects. We utilized the method of Sakurada et al. to measure regional cerebral blood flow (rCBF) in conscious rats. Male rats were fitted with a single ICV cannula, adapted to mild restraint over several weeks, and assigned to one of three groups: (Veh)-vehicle injection, H_2O available; (W+)-AII (100ng in 2 µl), H_2O available; (W-)-AII, H_2O available from injection to first lick only. Resulting rCBF data fell basically into 3 groupings: Gp 1) Relative to Veh, both W+ and W- exhibited significant increases in rCBF. This putative generalized drug effect was seen in such regions as the median preoptic, lateral and medial preoptic areas and cingulate cx; Gp 2) Increases in W- over both W+ and Veh, perhaps reflecting AII's action as a dipsogen. These areas included the organum vasculosum lamina terminalis, subfornical organ and paraventricular hypothalamus; Gp 3) A sequential increase in W+ and W- This group included the lateral and anterior hypothalamus and zona incerta. No decreases in rCBF were ever observed in any region or after any treatment. These data indicate that rCBF may be a sensitive measure for the detection of the central sites of action of AII as a dipsogenic agent, and may reveal distinctions between regions associated with drinking initiation and those reflecting motivation circuitry. (Supported in part by a grant from Marquette University graduate school dean).

510.13

SHAM-DRINKING BEHAVIOR IN RATS VARIES OVER THE DIURNAL LIGHT/DARK CYCLE. D. S. Dracos and F. W. Flynn. Dept. of Psychology and Neuroscience Program, University of Wyoming, Laramie, 82071.

Rats' intakes and 2-bottle preferences for sucrose and QHCl vary over the 12:12 LD cycle (Dracos & Flynn, 1989). Sham drinking tests were run to separate the role of oral from postoral effects on diurnal preference differences. Rats were entrained to a 12:12 LD cycle and fitted with gastric fistulae. At the onset of either the light (Light Group, n=5) or dark cycle (Dark Group, n=4), rats were presented with 4 ascending concentrations of sucrose, NaCl, HCl, and QHCl, and allowed to sham drink for 30 min or 1 h. The Dark Group sham drank significantly more 0.3 M sucrose (87.3 $\pm$ 12.4 ml) and 1.0 M sucrose (78.8 $\pm$ 7.0 ml) in 1 h than did rats in the Light Group (40.4 $\pm$ 5.9 ml and 41.6 $\pm$ 11.0 ml respectively), p's <0.05. In comparison to the Light Group, the Dark Group sham drank significantly more 0.01 M NaCl but significantly less of 0.03 M, 0.1 M, and 0.3 M NaCl, p's <0.05. There were no group differences in response to HCl. The Dark Group drank more 3 x 10-3 M QHCl than the Light Group, $p<0.05$. These results demonstrate that rats' sham intakes are influenced by the changes in physiology that accompany the 12:12 LD cycle and suggest that taste processing could be affected by the diurnal cycle. (Supported by NS-24879 awarded to F. W. Flynn.)

510.14

DIPSOGENIC STIMULI PAIRED WITH EATING SUPPORT CONDITIONED INITIATION OF DRINKING WITHOUT CONDITIONED WATER INTAKE IN RATS. F. S. Kraly and K. A. Spiess*. Dept. of Psychology, Colgate University, Hamilton, NY 13346.

The claim (Fitzsimons & LeMagnen, JCPP 67:273, 1969) that rats learn to associate orosensory properties of food with postprandial dehydrational consequences remains unexamined. We have done experiments in which an ingested food conditioned stimulus (CS) is paired with one of various dipsogenic unconditional stimuli (UCS; i.p. 0.5% BW 1M NaCl, s.c. 0.25 mg/kg angiotensin II (ANG), 5 mg/kg histamine or 0.9% NaCl as a control) known to be putative signals for drinking elicited by eating. The CS-UCS pairings on 2, 6 or 9 trials were followed by an extinction trial (i.e., CS without UCS) to examine for conditioned drinking. With a 30-min CS-UCS interval, the ANG UCS supported a conditioned aversion ($p<.001$) to a liquid food CS without evidence ($p>.10$) for conditioned drinking; the histamine UCS supported neither conditioned aversion nor water intake ($ps>.20$). With a 10-min CS-UCS interval, the 1M NaCl and histamine UCSs each supported conditioned initiation of drinking, measured by shorter ($ps<.05$) latency to initiate drinking upon presentation of CS on the extinction trial (vs. baseline) without supporting conditioned water intake ($ps>.10$). These results show that (a) two putative signals for food-related drinking, cell dehydration and histamine, serve as UCSs for conditioned initiation of drinking in response to a food CS; (b) conditioned water intake may be difficult to demonstrate.

510.15

DEHYDRATION- AND DEPRIVATION-INDUCED CHANGES IN NEURAL METABOLIC ACTIVITY IN THE HINDBRAIN OF 6-DAY-OLD RAT PUPS: IMAGE AVERAGING AND IMAGE DIFFERENCING OF 2-DG AUTORADIOGRAMS. S.E. Swithers and W.G. Hall. Department of Psychology, Duke University, Durham, NC 27706.

In 6-day-old rat pups, both dehydration and overnight deprivation enhance ingestion. The modulatory effects of dehydration and deprivation may have similar neural bases, or may be accomplished by different neural mechanisms. To examine these possibilities, the topography of neural metabolic activity during a 1 hour [14-C] 2-deoxyglucose (30 uCi/100g BW, s.c.) incorporation period was compared in 6-day-old rat pups that were cellularly dehydrated by injection of hypertonic saline or that were 24 hours deprived. Autoradiographic images corresponding to sections of the neural axis at several levels of the hindbrain were selected for each experimental group and analyzed. Difference images were then generated by subtracting average images from one another.

Relative to 24 hour deprived pups, cellularly dehydrated pups showed increased metabolic activity in parabrachial nuclei, nucleus of the mesencephalic trigeminal nerve, oral regions of the nucleus of the spinal trigeminal nerve, nucleus ambiguus, and areas between sensory and motor trigeminal nuclei. Cellularly dehydrated pups had decreased activity in the oral region of the nucleus of the solitary tract as well as in medullary and pontine reticular areas.

510.16

SHORT TERM FOOD DEPRIVATION CAUSES PICA PROPORTIONATE TO THE AMOUNT OF FOOD WITHHELD. D. Mitchell. Psychology Dept., Univ. of Southern California, L.A., CA 90089-1061.

Geophagy (clay consumption) is a common form of pica (consumption of nonnutritive substances) during famines. Rats will frequently engage in appreciable amounts of pica during brief periods (23 hr) of food deprivation. Previous experiments employing emetic toxins, motion sickness, and conditioned illness indicate that pica relieves the subjective symptoms of gastrointestinal malaise. Assuming that over short deprivation periods the more food withheld, the more severe or prolonged the unpleasant gastrointestinal sensations, the present experiment determined if pica shows a systematic relationship to the amount of food withheld. Accordingly, rats maintained with water and clay (kaolin) continuously available were subject to periodic episodes of food restriction in a counterbalanced Latin Square design. Thirty animals that had consumed at least 15.0 g of kaolin on a 23 hr food-deprivation screening test were matched and randomly assigned to six groups. Each group was tested in a unique sequence of conditions spaced six days apart during which they were permitted 23 hr access to 0, 5, 10, 15 20 g or ad lib food. They consumed 19.9, 10.1, 7.6, 5.3, 3.2, and 0.2 g of kaolin respectively. These results indicate that pica induced by food deprivation is similar to other causes of pica and that it is mediated by subjective visceral sensations.

510.17

HUMAN SATIETIN (h-SAT) PURIFIED ON HPLC INFUSED INTRACEREBROVENTRICULARLY (ICV) DOES NOT PRODUCE CONDITIONED TASTE AVERSION IN RATS. L.L. Bellinger and V.E. Mendel. Depts. Physiol. Baylor Coll. Dent., Dallas, TX 75246 and Animal Physiol. Univ. Calif., Davis 95616.

Previously (Pharm Biochem & Behav 23:559, 1985) we found semi-purified (sp) h-SAT produced taste aversion in a two bottle test when infused ICV. In the present study sph-SAT was purified on HPLC, which yielded two peaks (P-A, 10.7%; P-B, 89.3% by wt.) Male Sprague Dawley rats (327-404g) were fitted with chronic third ventricle cannulas. The rats (LD 12:12, light out at 1130h) were given access to water in calibrated bottles, for 1 h/day (1030-1130h) and food ad libitum for 4 weeks. The rats were then divided into three groups (GRP): GRP 1, control (n=8); GRP 2, P-A (n=11); GRP 3, P-B (n=10). On day 1 all groups were ICV infused (10 μl) with artificial-cerebrospinal fluid (a-CSF) at 1000 h. At 1030 h, the control group was given almond flavored (0.5% extract) water (AFW) and the experimental groups banana flavored water (BFW). One hour and 24 h food intake (FI) and 1 h fluid intake were measured and found to be similar in all groups. On the next day the groups were ICV infused with: GRP 1, a-CSF and given BFW; GRP 2, P-A, 11 μg/rat and GRP 3, P-B, 90 μg/rat; both groups were given AFW. Fluid intake was similar in all groups, however, 1 h and 24 h FI were both suppressed ($P<0.05$) in GRP 3 (1.4$\pm$0.3g, 11.2$\pm$0.8g) compared to GRP 1 (2.6$\pm$0.5g, 16.0$\pm$1.0g) and GRP 2 (3.0$\pm$0.3g, 14.8$\pm$1.2g). On day 4 the groups were given a two-bottle choice test of AFW vs BFW, GRP 1, 7.1$\pm$2.1 vs 11.0$\pm$1.4 mls; GRP 2, 6.9$\pm$0.9 vs 6.9$\pm$1.3 mls; GRP 3, 9.2$\pm$1.7 vs 6.9$\pm$1.8 mls, all N.S.). These data suggest HPLC purified P-B can suppress FI without producing taste aversion and thus may be a physiological satiety agent.

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510.18

CONDITIONED FOOD AVERSIONS INDUCED BY CHRONIC LITHIUM CHLORIDE INFUSIONS IN RATS: EFFECTS OF AREA POSTREMA LESIONS. L.A.Eckel* and K.-P. Ossenkopp. Dept. Psychology, Univ. Western Ontario, London, Ontario, CANADA N6A 5C2.

Lithium has been shown to be a highly effective agent in inducing conditioned food aversions (CFA). The effects of chronic LiCl infusion on food intake and diet preference in rats with area postrema lesions (APX) or sham lesions (APS) were examined. Osmotic minipumps, filled with a saturated aqueous solution of LiCl, were implanted into the peritoneal cavity of APX and APS rats. Similarly, nonfunctional pumps were implanted in two other groups of APX and APS rats. The LiCl infusion or sham drug phases were paired with free access to a novel diet during a 7 day conditioning phase. Food intake was measured during the conditioning phase and preferences for the novel diet vs. a familiar diet were assessed a week after the conditioning phase. During the conditioning phase the APS group infused with LiCl exhibited a significant reduction in food intake relative to the sham drug APS group ($p < .01$). No significant differences in food consumption were found between the two APX groups. Group APS given LiCl also showed a marked aversion to the novel diet, whereas group APX given LiCl did not. These results indicate that CFAs induced with chronic LiCl infusions are mediated by the area postrema. (Supported by a NSERC grant to KPO).

510.19

EFFECTS OF ZACOPRIDE AND 8-OH-DPAT ON BODY ROTATION-INDUCED CONDITIONED TASTE AVERSIONS IN RATS. L.A.Ruttan and K.-P. Ossenkopp. Dept. Psychology, Univ. Western Ontario, London, Ontario, CANADA N6A 5C2.

The 5HT-3 antagonist zacopride and the 5HT-1a agonist 8-OH-DPAT have both been shown to possess anticmetic properties. Two separate experiments examined the effects of zacopride and 8-OH-DPAT on body rotation-induced conditioned taste aversions (CTA) in male hooded rats. The rats were adjusted to a 23.5 h/day water deprivation schedule. On 3 conditioning days 30 min access to a 0.1% sodium saccharin solution was followed by subcutaneous administration of either zacopride (0.1 mg/kg), 8-OH-DPAT (0.1 mg/kg), or isotonic saline. Fifteen minutes following drug administration the animals were exposed to the body rotation procedure which consisted of 30 min rotation at 70 rpm on a schedule of 60 s on - 60 s off. The animals were again given access to water (30 min) on each of the next 4 days and were then tested for saccharin preference (two-bottle tests) over the next 12 days. The results indicated that administration of 8-OH-DPAT significantly enhanced the body rotation-induced CTA ($p < .01$), whereas zacopride significantly attenuated the induced CTA ($p < .05$). (Supported by a grant from NSERC to KPO).

510.20

IBOTENIC ACID LESIONS OF THE LATERAL HYPOTHALAMUS BLUNT AFFECT BUT DO NOT INDUCE TASTE AVERSION. K.C. Berridge, The University of Michigan, Department of Psychology, Ann Arbor, MI, 49109.

Aphagia and increased aversive taste reactivity (gapes, etc.) occur after electrolytic lateral hypothalamic lesions (Teitelbaum & Epstein, '62; Schallert & Whishaw, '78; Stellar et al., '79; Fluharty & Grill, '81) and after excitotoxic striatopallidal lesions (Berridge & Cromwell, in press). Aversion is not enhanced, in contrast, after nigrostriatal 6-OHDA lesions that produce aphagia (Berridge et al, '89). Do *excitotoxic* lesions of the lateral hypothalamus that produce aphagia increase aversion to tastes? Thirty-two male rats received bilateral injections of ibotenic acid (0.8 ul, 1.2 M) into the lateral hypothalamus (A-2.0,L±1.9,V-8.0). Ten rats showed aphagia that lasted at least 8 days after the lesion. Nine control rats received vehicle injections. Taste reactivity to 1 ml oral infusions of sucrose, NaCl, citric acid, and quinine solutions (delivered via chronic oral cannulae) was videotaped and assessed frame-by-frame.

Aphagic rats showed reduced levels of aversive *and* hedonic reactions (tongue protrusions, etc) to tastes, compared to control rats or to rats that were not aphagic after lesions. Although a reduction of sensorimotor arousal might explain this result, sensorimotor deficits have been argued *not* to be produced by excitotoxic hypothalamic lesions (Dunnett, et al, '85). An alternative explanation is that a global *blunting* of hedonic and aversive affect is produced by the loss of intrinsic neurons from the lateral hypothalamus. Enhancement of aversion by electrolytic lesions may require a synergistic destruction of multiple hypothalamic elements. Alternatively, enhanced aversion may require destruction of neurons from bordering regions that are outside of the hypothalamus itself.

510.21

TASTE AVERSION LEARNING IN THE FERRET: LIMITS ON CONDITIONED AND UNCONDITIONED STIMULI. B. M. Rabin and W. A. Hunt*. Armed Forces Radiobiology Research Institute, Bethesda, MD 20814, and University of Maryland Baltimore County, Baltimore, MD 21228.

Previous research has shown that treatment with toxic stimuli that produces vomiting also produces conditioned taste aversion (CTA) learning at a lower dose. To further evaluate the relationship between emesis and CTA learning, ferrets were allowed to drink a 10% sucrose solution immediately prior to injection of lithium chloride (LiCl) or exposure to ionizing radiation. Treatment with neither unconditioned stimulus resulted in the acquisition of a CTA, even when vomiting was produced by the toxin. In contrast, when canned cat food was used as the conditioned stimulus, a CTA was produced by injection of LiCl (3.0 mEq/kg), but not by exposure to radiation (200 cGy). These results, in relation to those of other studies, indicate that the association between the CTA and emetic effects of exposure to toxins may be species specific, depending upon the nature of both conditioned and unconditioned stimuli.

LEARNING AND MEMORY: SPATIAL

511.1

A NEW METHOD FOR ESTIMATING SPATIAL LOCALIZATION IN RATS. A. Speakman* & J. O'Keefe*. (SPON: Brain Research Association) Anatomy Dept, University College, London WC1E 6BT UK

A widely used apparatus for estimating spatial localization in studies of hippocampal function is the 'water maze' (Morris, R.G.M., Learn. Motiv., 1981, 12:239). We describe a task which is similar in principle, but which has the advantage that the rat is not immersed in water. Instead the rat is motivated by appetite to approach a goal defined only by its spatial relationship to distal cues.

A small skull-mounted post is chronically implanted in a rat. During training an apparatus is attached with a nozzle which sits in front of the animal's mouth during free behavioural movement across a circular arena (1.18m diameter). A length of plastic tubing connects the dispensing apparatus to a distant solenoid valve and pressurised liquid reservoir. Operation of the solenoid valve results in a squirt of saccharine solution directly into the animal's mouth. Typically a volume of 0.1ml saccharine at a concentration of 0.4% is dispensed over 100ms. A computer is used to track the animal's position and to administer the reward at particular places (goals).

We have shown that rats will learn to approach goals defined in this manner. In addition, by rotating controlled cues from trial to trial, we have shown that distal cues can control spatial localization of this type. Two methods have been used to gauge the accuracy of spatial localization in relation to the cues. In a 'titration' method, rats received reward over a particular spatial area which gradually shrank/expanded as the rat demonstrated it could/could not find the goal. In the 'radial reward' method, the probability density of reward varied as an inverse function of the distance from the goal.

511.2

DISCRETE EXCITOTOXIC LESIONS OF THALAMUS DO NOT DISRUPT PERFORMANCE OF RATS ON A SPATIAL DELAYED NON-MATCHING TO SAMPLE (DNMTS) TASK. J.K. Robinson, S. Koger, D.M. Lacourse*, and R.G. Mair. Dept. Psychol., Univ. New Hampshire, Durham, NH 03824.

To study the role of thalamic lesions in DNMTS deficits caused by pyrithiamine treatment (NSci Abst 15:304, 1989), 48 rats were pretrained on DNMTS and given one of four treatments: midline thalamic lesion, (N=16) bilateral thalamic lesion (±1mm from midline) (N=16), sham lesion of hippocampus overlying the bilateral site (N=8), and sham surgery (N=8). The injections of 5µl of 1 mM NMDA destroyed tissue within a radius of .45 mm of the cannula. None of the lesioned animals were impaired on measures of response accuracy or speed at any of the memory delays tested. These data contrast with those of more extensive RF lesions of thalamus (cf Mair, et al, this meeting).

511.3

DIENCEPHALIC ANTEROGRADE AMNESIA FOR SPATIAL INFORMATION IN THE RAT. R. J. Sutherland, H. N. Rice* and J. M. Hoesing. Dept. of Psychology, The Univ. of Lethbridge, Lethbridge, AB, Canada, T1K 3M4.

The necessary subcortical damage for "diencephalic" amnesia is not established. We sought to determine: 1. if prelesion training on the "procedural" aspects of the Morris water task would ameliorate the deficit in new place learning associated with thalamic or mammillary damage and 2. the relative magnitude of impairment associated with damage to anterior thalamic (AT), mediodorsal thalamic (MD), and/or mammillary bodies (MB).

All rats were trained preoperatively to swim to a hidden platform in the Morris water task. Each day the platform was positioned in a new location in the pool. They were divided into 5 groups receiving electrolytic lesions to MD, AT, MB, MD+MB, or sham lesions. All rats were retested in the moving platform version of the Morris water task. Neither MD nor MB damage alone reliably impaired performance. Rats with AT or MD+MB damage showed an inability to learn the hidden platform location throughout postoperative testing. The results provide support for the idea that combined damage to circuitry involving MD and MB is necessary to produce a severe diencephalic anterograde amnesia. Interestingly, performance in this task is not sensitive to amygdala damage.

511.4

MEDIAL SEPTAL LESIONS DISRUPT SPATIAL, BUT NOT NONSPATIAL, WORKING MEMORY IN RATS. John E. Kelsey and Hannah Vargas*, Dept. Psychology, Bates College, Lewiston, ME 04240

Damage to the projection from the medial septal nucleus (MS) to the hippocampus has been hypothesized to disrupt spatial working memory more than it disrupts nonspatial working memory (Aggleton, Hunt, & Rawlins, 1986). To further test this hypothesis, rats with MS lesions were first trained on a spatial delayed nonmatching to sample (DNMTS) task in a Y-maze and then on a nonspatial DNMTS task in the same Y-maze. The spatial task required the rats to enter the arm opposite that which they were forced to enter on the preceding run in order to obtain .5 cc of 8% sugar water. As anticipated, rats with small MS lesions were less accurate in selecting the opposite arm during acquisition when the retention interval was 10 sec. Moreover, in subsequent tests when the retention interval was varied between .5, 1, and 2 min, the deficit increased as the retention interval increased. The nonspatial task required the rats to enter the arm containing the object not encountered in the straight alley on the preceding forced run. In contrast to the spatial task, MS lesions did not disrupt accuracy of choice during acquisition or during subsequent tests when the retention interval was varied. Although it is possible that the difference in the effects of the lesions on these two tasks could be due to differences in task difficulty or to recovery of function, these possibilities seem unlikely. Thus, these results suggest that rats with small MS lesions have more difficulty remembering where they have been than they do remembering what object they have just seen.

511.5

IMPAIRED SPATIAL MEMORY FOLLOWING VENTROMEDIAL THALAMIC LESIONS IN RATS. J. D. Butler and D. B. Neill. Dept. of Psychology, Emory University, Atlanta, GA 30322.

Neuroanatomical and electrophysiological experiments indicate that the ventromedial nucleus of the thalamus (VMT) may have a nonspecific arousal regulatory effect on neocortex. Observations from past experiments in our laboratory lead us to believe that the VMT may participate in memory processes. We have found that rats bearing electrolytic lesions of VMT are impaired in the acquisition, but not the retention, of a number of conditioned responses. In the present experiment, we evaluated the ability of VMT rats to perform in a commonly-used test of "working" memory, the radial arm maze. Ten naive, male Sprague-Dawley rats were trained to retrieve food from the arms of a 8-arm radial maze. Each daily test consisted of a study phase in which four arms of the maze were blocked. The rats retrieved food from the non-blocked arms and were then removed from the maze for 45 sec. During this interval the remaining arms of the maze were opened. Upon reintroduction to the maze (retention phase), the task was to retrieve food from the previously unvisited closed arms. Errors were defined as entrances to arms visited previously in either the study or retention phases. Upon reaching criterion, the rats were then divided into two groups of 5 animals each. The VMT group received anodal electrolytic lesions (1 microamps for 10 sec) in the VMT. In the sham operated group the electrode was lowered but no current was passed. The memory test was then administered once daily for 9 days. VMT lesioned animals displayed significantly more errors than did sham operated rats.

Supported by the Emory University Research Fund.

511.6

HIPPOCAMPAL LESIONS IN RATS PRODUCE A TEMPORALLY-GRADED RETROGRADE AMNESIA ON A SPATIAL MEMORY TASK. J.L. Kubie, S. Dayyani, R.U. Muller, B. Cohen, E. Major and R.J. Sutherland. S.U.N.Y. Health Sci Center, Brooklyn NY and Univerisity of Lethbridge, Alberta, CAN

A temporally-graded retrograde amnesia is a characteristic of the human amnestic syndrome. A recent water-maze study by Sutherland *et al* suggests a parallel in rats: Animals trained 14 weeks before hippocampal lesions were able to reacquire efficient behavior while those trained immediately before surgery were seriously impaired on reacquisition (Neurosci Abstr **13**:1066, 1987).

In a study reported last year we attempted to further investigate this phenomenon with another spatial task -- an appetitive task run in a dry, cylindrical enclosure (Kubie *et al*, Neurosci Absrt **15**:609, 1989). We found that rats trained 14 weeks before surgery had no retrograde loss -- they rapidly reaquired the spatial task. In contrast, the same rats exhibited an anterograde amnesia in that, after hippocampal lesions, they could not learn to navigate towards a second, novel location. The current study is an extension of our previous work and asks two questions: Is there evidence of temporally graded retrograde amnesia? and, if so, is the retrograde amnesia specific to spatial memories?

In the first experiment, using procedures identical to the earlier study, rats were trained on the spatial task within a two week interval, and were then immediately subjected to hippocampal (n=5) or sham (n=6) lesions. After sufgery rats with hippocampal lesions were severely impaired, and 4 never reacquired spatial abilities on the spatial task. This is in contrast to previous data where rats with a 14 week interval between training and surgery were relatively unimpaired. Thus, there is support for a temporal gradient in the retrograde amnesia.

In the second experiment, rats were trained in an object discrimination task where each rat was rewarded for digging below one out of 12 objects in an open camber. Following a 2-week acquisition period rats were given hippocampal (n=3) or sham (n=1) lesions. After recovery, all rats showed perfect retention of the preoperative habit. Thus, the retrograde amnesia seen in rats appears to be at least somewhat specific to spatial memory. (supported by NIH grant RO1-NS20686)

511.7

SPATIAL AND CUED DISCRIMINATIONS: ROLE OF THE HIPPOCAMPUS AND PROTEIN KINASE C S.Golski, D.S. Olton, M.Mishkin, J.L.Olds, and D.L.Alkon. Dept. of Psychology, Johns Hopkins University, Baltimore, MD 21218.

The role of the hippocampus in spatial and cued discrimination learning was examined in two experiments in a modified, cue-controlled water maze: (1) the effects of hippocampal lesions on performance, (2) the effects of performance on protein kinase C (PKC) in the hippocampus. The spatial discrimination (SD) and cued discrimination (CD) were equivalent in terms of the sensory and motor components, but differed in the stimuli that were relevant and irrelevant to the discrimination. Hippocampal lesions (HPC) impaired performance relative to controls (CON) in the SD, but not the CD. Mean response time (and standard error) to reach the submerged platform on the final block of trials was: SD-CON=5.4(0.6), SD-HPC=12.6(1.6), CD-CON =3.2(0.3), CD-HPC=5.1(1.1)(N=8, each group). Alterations in PKC were determined by autoradiography in separate groups of rats after 2 or 9 sessions of training (8 trials per session) in either discrimination.

511.8

DIFFERENTIAL EFFECTS OF MEDIAL SEPTAL LESIONS ON ACQUISITION IN TWO SPATIAL MEMORY TASKS. M.W. Decker, R. J. Radek* and M. A. Pelleymounter, Neuroscience Research, Abbott Labs, Abbott Park, IL 60064.

Medial septal lesions disrupt cholinergic input to the hippocampus and produce behavioral deficits similar to those observed following hippocampal lesions. In the current study, however, medial septal lesions differentially affected acquisition of two spatial memory tasks generally regarded as being sensitive to hippocampal damage--the radial arm maze and the Morris water maze.

Radiofrequency lesions of the medial septum (MS) were made in male, Long-Evans rats. These rats and nonoperated controls were then trained in a Morris water maze to find a camoulflaged escape platform located in a fixed position across trials. Escape latencies for MS-lesioned and control rats were not significantly different, and anlaysis of search patterns during a probe trials conducted in the absence of an escape platform revealed no differences between groups. When these rats were later trained on the radial arm maze, however, the MS-lesioned rats displayed marked impairments that persisted throughout a 13-day training experience ($p<.001$). Thus, these MS lesions impaired spatial learning in the radial arm maze, but not in the water maze.

Experiments with a different set of rats revealed MS lesion-induced impairment of short term, within session habituation of locomotor activity in an open field, but no effect on habituation of activity observed across days. This finding may be related to the differences observed between the effects of these lesions on the radial maze and the water maze since information regarding the platform location in the water maze is typically maintained over longer periods than is information regarding arms visited in the radial maze. The distinction, however, does not appear to be related to arbitrary, experimenter-imposed working /reference memory manipulations, as further work revealed no lesion effect on the performance of a "working memory" version of the water maze in which a new platform location was used each day.

511.9

ROLE OF THE HIPPOCAMPUS AND POSTERIOR PARIETAL CORTEX IN EXPLORATION AND RESPONSE TO A SPATIAL CHANGE IN RATS. C. Thinus-Blanc, B. Poucet, E. Save* and M.C. Buhot*. Lab. Functional Neurosci., C.N.R.S., 31 chemin Joseph-Aiguier, 13402 Marseille, France.

This study was aimed at dissociating the role of the posterior parietal cortex (PPC) and hippocampus (HPC) in exploration and in reaction to spatial novelty. In two experiments, rats were first allowed to explore an open field containing one or several objects (habituation phase). Then a change was brought to the initial situation either by removing the object (Experiment 1) or displacing two objects among five (Experiment 2). Both changes could be detected on the basis of the spatial array only. The behavioral reaction to the change was measured by the time spent exploring.

Rats with PPC lesions or HPC lesions were tested. We also investigated the effect of reversible short-lasting inactivation of the hipppocampus in rats chronically equipped with cannulae implanted into the ventral hippocampus and injected with lidocaine. By comparison with control rats who spent more time at the location of the disappeared stimulus (Experiment 1) or who reexplored selectively the displaced objects (Experiment 2), the hippocampally damaged rats did not display any reaction to change in either experiment. As hippocampal rats, animals with PPC lesions did not react to the change in Experiment 1. In contrast, in Experiment 2, rats with PPC lesions reexplored the whole set of objects. Therefore, the role of PPC appears to be more subtle than that of HPC. PPC may have a particularly important role in discriminating spatial environmental changes that result from the absence of familiar visual cues.

511.10

EFFECTS OF A SHORT-LASTING REVERSIBLE INACTIVATION OF THE HIPPOCAMPUS AND SEPTUM ON SPATIAL LEARNING IN RATS. B. Poucet, M.C. Buhot* and C. Thinus-Blanc. Lab. Functional Neurosci., C.N.R.S., 31 chemin Joseph-Aiguier, 13402 Marseille, France.

The present study was aimed at testing the effects of a reversible inactivation of either the hippocampus or the septum on long-term and short-term spatial learning in the rat. In a circular platform with 18 holes on the periphery, rats chronically equipped with cannulas into the ventral hippocampus or the septum were trained to locate the unique hole leading to a hidden shelter in order to avoid a bright light. In Exp.1, the task emphasized long-term acquisition of spatial information (1 trial/day for 16 days, 24 hrs ITI). The location of the hole was changed on the 17th day and rats were injected with lidocaine just before each of the further 8 daily trials. Both lidocaine-injected and sham-injected rats relearned the new location at a similar rate. In Exp.2 which emphasized short-term acquisition of spatial information (3 trials/day, 1min ITI), rats were sham-injected or lidocaine-injected on alternate days and had to learn a new hole location each day. While sham-injected rats improved in orientational accuracy over successive trials, lidocaine-injected rats did not. These results confirm the role of the septum and hippocampus in spatial learning. However, rats were impaired only when septo-hippocampal activity was neutralized over the whole course of spatial processing (Exp.2). In contrast, inactivation of the septo-hippocampus during restricted periods of a longer-term process did not seem to prevent normal function in spatial learning (Exp.1). It is hypothesized that the septo-hippocampal formation could process information "off-line" in the delay between two learning trials.

511.11

FIMBRIA-FORNIX TRANSECTIONS DISRUPT THE ONTOGENY OF DELAYED ALTERNATION BUT NOT POSITION DISCRIMINATION IN THE RAT. M.E. Stanton and J.H. Freeman Jr. Neurotox. Div., U.S. EPA, Research Triangle Park, NC 27711 and Dept. of Psychology, UNC-Chapel Hill, Chapel Hill, NC 27514.

Three experiments examined the effects of fimbria-fornix transections on the ontogeny of discrete-trials delayed alternation (DA) and position discrimination (PD) in a T-maze (for procedures see Green & Stanton, Behavioral Neuroscience, 1989, 103, 98-105). In Experiment 1, 23-day-old rat pups given lesions on postnatal day 10 (PND10) acquired PD, but not DA after 60 training trials. Experiment 2 showed that this same lesion prevented the developmental emergence of DA between PND19 and PND27. Experiment 3 showed that 23-day-old pups with these lesions begin to show acquisition of DA, if given 72 additional training trials. Lesions were verified by histological examination of the fornix and AChE staining in the hippocampus.

The septohippocampal projection appears to be necessary for the ontogeny of DA but not PD in the rat. The lack of effect on PD suggests the lesion selectively impairs memory processes subserving DA. These findings suggest a role for septohippocampal maturation in the ontogeny of these memory processes.

511.12

NEONATAL MEDIAL PREFRONTAL CORTEX LESIONS AND SPATIAL DELAYED ALTERNATION IN THE DEVELOPING RAT: RECOVERY OR SPARING? J.H. Freeman Jr. and M.E. Stanton. Dept. of Psychology, UNC-Chapel Hill, Chapel Hill, NC 27514 and Neurotox. Div., U.S. EPA, Research Triangle Park, NC 27711

Two experiments examined the effects of medial prefrontal cortex aspiration lesions on acquisition of T-maze delayed alternation (DA) and position discrimination (PD) in infant rats (see Green & Stanton, Behavioral Neuroscience, 1989, 103, 98-105, for procedural details). In Experiment 1, rat pups given lesions on postnatal day 10 (PND10) and trained on PND23, acquired PD, but not DA after 60 training trials. Experiment 2 showed that this lesion-induced impairment was no longer evident in animals trained on PND33. Lesions were verified by histological examination of cresyl-violet stained brain sections.

The medial prefrontal cortex appears to be involved in DA but not PD during early development in the rat. The differential effect on these two tasks suggests a selective impairment of the memory processes subserving DA. The failure of this early lesion to impair DA learning in adult rats therefore appears to reflect recovery rather than sparing of function (Kolb & Nonneman, Brain Res., 1978, 151, p. 144). This recovery may occur between 23 and 33 days of age in the rat.

511.13

CEREBRAL ^{14}C-GLUCOSE UPTAKE PATTERNS INDUCED IN MICE BY SPATIAL DISCRIMINATION TESTING IN AN EIGHT ARM RADIAL MAZE.
B. Bontempi*, J. Sif*, C. Messier, R. Jaffard and C. Destrade. Lab. Psycho- physiol. URA CNRS 339, Univ. Bordeaux I, 33405 Talence France and (J.S.) Biol. Dept, BP 20 El Jadida Univ. Marocco.

Regional mapping of ^{14}C-glucose (GLU) uptake was analyzed in mice at different time intervals both the first (Day 1) and last (Day 9) daily sessions of a spatial discrimination testing procedure in an eight-arm radial maze. BALB/c mice with a jugular catheter were randomly divided into four groups: two experimental groups, respectively trained for 1 day and 9 days in the spatial discrimination task and two (quiet and active) control groups. Each experimental group was divided into three subgroups injected with GLU at different post-training (PT) intervals (30 s, 1 hr and 3 hr); the animals were sacrificed 5 min later and the brain processed for autoradiography. The mapping of GLU was analyzed using the relative glucose uptake method. On Day 1, a progressive increase of labelling was found in trained animals for the medial septum and the mediodorsal thalamic nucleus (5 min PT), the hippocampal formation (1 hr-PT) and the subiculum and the frontal cortex (3 hr-PT). In contrast, on Day 9, increased labelling was found 5 min-PT in all previously mentioned regions. However, 1hr and 3 hr-PT, no significant labelling was found in these structures.

These results suggest that the sequential nature of the activation observed on Day 1 represents the time-dependent progressive organization of memory.

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511.14

DIFFERENTIAL EFFECTS OF MEDIODORSAL THALAMIC OR MAMMILLARY BODIES IBOTENIC ACID LESIONS ON SPATIAL SHORT-TERM MEMORY IN MICE.
F. Alaoui-Bouarraqui *, D. Béracochéa and R. Jaffard. Lab. Psychophysiologie, URA CNRS 339, Univ. Bordeaux I, 33405 Talence Cedex France.

Some of the deficits observed in the human Korsakoff syndrome (impairments of temporal order judgements, exagerated vulnerability to interference) have been attributed to a frontal lobe dysfunction even though direct damage to the frontal cortex of these subjects is not systematic. Given the constant presence of diencephalic damage in Korsakoff subjects, it remains possible that these diencephalic damage are involved in frontal-related deficits. In the present experiments, we have studied the effects of lesionning either the mediodorsal thalamic nucleus (MD) or the mammillary bodies (MB) on memory tasks known to reveal impairments resulting from frontal lesions.

The tasks were run in an automated 8-arms radial maze. In the **"recognition task"**, the basic procedure relied on a forced visit (target-visit) followed immediately by a test-phase during which the subject had to choose between the target-visit and a new , non-visited arm (win-shift strategy). The difficulty of the task was increased by including interfering forced visits (from 1 to 5 i.e. study-phase) between the target-visit and the test-phase. This task was designed to study the vulnerability to interference. In the **"serial-order task"**, all arms were visited during the study-phase; the position of the target-visit was varied, from the 1st to the 6th one. During the test-phase, the subject had to choose in all cases between a given target-visit and the last visited arm of the study-phase. Thus, the difficulty of the recognition increased as a function of the recency of the target-visit. Results showed that MD lesions induced a marked deficit in the serial order task but not in the recognition one, whereas the opposite was observed following MB lesions.

These results provide evidence for a differential involvement of subcortical damage in frontal-related deficits.

511.15

SEX-SPECIFIC PATTERNS OF SPATIAL BEHAVIOR PREDICT HIPPOCAMPAL SIZE IN WILD RODENTS. L.F.Jacobs*S.J.C.Gaulin*,D.F.Sherry and G.E.Hoffman. Dept. of Anthropology,Univ.Pittsburgh,Pittsburgh,PA 15260.

In polygamous vole species males range more widely than females in the field and perform better on laboratory measures of spatial ability; both differences are absent in monogamous vole species. Such cognitive differences, predicted by theories of sexual selection, should be reflected in the size of the hippocampus, a structure known to mediate spatial learning and whose size, in inbred mouse strains, correlates positively with maze performance.

Ten females and males were taken from wild populations of two vole species. The volume of the hippocampus, relative to the entire brain, was determined from serial brain sections. Males of the polygamous species, Microtus pennsylvanicus,had significantly larger hippocampi than did females, whereas there was no sex difference in hippocampal size in the monogamous species, M. pinetorum. Our result suggests that the structure of functional subunits in the brain can be shaped by evolutionary processes to meet particular cognitive demands. In Microtus, the pressures of sexual selection may have produced adaptive sex differences in brain structure and behavior.

511.16

THE ONTOGENY OF PLACE MEMORY IN THE RAT AND INFLUENCES OF REARING IN AN ENRICHED ENVIRONMENT. J. R. Keith, J. W. Rudy* and J. P. Terrones. Dept. of Psych., Univ. of Colorado, Boulder, CO 80309.

Rats are capable of place learning by the third postnatal week (Rudy et al., Behav Neurosci, 101, 1987). Yet place learning and memory are dependent upon several cortical structures that continue to mature after the third postnatal week. So, one might expect further improvement in some of the processes that mediate place learning after Day 21. This hypothesis was evaluated by determining if there are changes during the postweaning period in rats' ability to retain place information.

Young animals (21 days old) were impaired, relative to older animals (40 days old) at learning the hidden-platform version of the Morris navigation task when a training schedule was used which required animals to retain and build upon information provided over long intervals. Young animals also forgot a well-learned hidden-platform problem more rapidly than older animals. Yet young animals retained a proximal cue-based version of the task for up to 120 h suggesting that their failure to retain the hidden--platform task reflected a selective inability to retain distal-cue-based place information.

Rearing animals in an enriched environment accelerated the maturation of the animals' ability to retain place information but had no effect on acquisition of place information.

511.17

AMPA- AND IBOTENATE-INDUCED LESIONS OF THE BASAL FOREBRAIN: DISSOCIABLE EFFECTS ON WATER MAZE ACQUISITION AND PASSIVE AVOIDANCE RETENTION. K.J. Page*[1], H.M. Marston*[2], L.S. Wilkinson*[2], T.W. Robbins[2] and B.J. Everitt[1]. Depts. of Anatomy[1] and Experimental Psychology[2], Univ. Cambridge, CB2 3DY, England.

Ibotenic acid or AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionate) was infused into the region of the nucleus basalis of Meynert (nbM) of rats. Acquisition and performance of a water maze over a 10-day period (2 trials/day) were unaffected by AMPA-induced lesions which caused an 80% decrease in cortical choline acetyltransferase (ChAT) activity. However acquisition, but not performance, was significantly impaired by ibotenate lesions, which caused a 60% reduction in cortical ChAT activity. Ibotenate-lesioned rats swam further in the training quadrant during a probe trial, when the platform was removed, than either AMPA-lesioned or control rats, suggesting a perseverative search strategy. Neither ibotenate, nor AMPA, lesions affected step-through passive avoidance (PA) acquisition. However, rats with AMPA, but not ibotenate, lesions were significantly impaired in a 96hr retention test. Histological analysis revealed that AMPA infusions destroyed more nbM ChAT-immunoreactive neurons than did ibotenate infusions but, unlike ibotenate infusions, spared the overlying globus pallidus and many parvocellular neurons of the ventral pallidum. The ibotenate-induced impairment in the water maze appears to be related to pallidal, not nbM, damage. The AMPA-induced impairment in PA retention appears to be more directly related to destruction of magnocellular cholinergic nbM neurons.

511.18

EFFECTS OF MEDIAL SEPTAL LESIONS ON ACTIVITY AND WATER MAZE PERFORMANCE. John J. Boitano, Carl P. J. Dokla, Sean Parker, Kristina Stalzer*, Nanci Norelli*, Melissa Fiorini*. Depts. of Psychology, Fairfield University, Fairfield, CT 06430, & St. Anselm College, Manchester, NH 03102.

The effects of electrolytic lesions of the medial septal area (MSA) in F-344 rats was examined using 2 activity measures and reversal learning in the Morris water task. In the circular open field, the number of areas entered by control and lesioned A's were contrasted over 8 days (5 min/day). In a closed field situation, all A's were given forced confinement in an empty goal box (40 mins over 4 days), and were then placed in the field for 5 daily sessions. Latency to head-poke into the goal box plus the areas traversed were converted to a velocity index. Both locomotion and velocity were significantly reduced in the MSA rats ($ps < .01$). Acquisition in the water maze over 16 days revealed insignificant group differences. In a test of reversal learning, one month later, MSA rats took significantly longer to locate the hidden platform (4 days, 2 trials/day; $p < .0003$). While the findings support previous research concerning hypoactivity in MSA A's, they raise unanswered questions about possible neuroanatomical changes taking place after acquisition.

511.19

BEHAVIORAL EFFECTS OF COMBINED CHOLINERGIC AND NORADRENERGIC LESIONS. D.J. Connor, P.J. Langlais and L.J. Thal San Diego VAMC and Dept. of Neuroscience, UCSD, La Jolla, CA.

To examine the behavioral effects of combined lesions of the cholinergic and noradrenergic systems, we lesioned the nucleus basalis magnocellularis (nBM) and dorsal noradrenergic bundle (DNB) of adult F-344 rats (280-300g) with ibotenic acid and 6-OHDA respectively. ChAT activity was significantly decreased from controls in both the nBM and nBM/DNB groups (depletion %ant./%post. = 40.5/20.2 and 33.6/15.2 respectively) and was nonsignificantly increased by the DNB lesion alone (5.6/4.3). NE levels were depleted by >90% by the DNB lesion with no effect of the nBM lesion on NE levels. Acquisition of the Morris water maze showed an impairment of performance by the nBM lesion and an independent enhancement of performance by the DNB lesion. Reversal of the task revealed a similar pattern with the DNB lesion decreasing the behavioral effects of the nBM lesion. A water-motivated T-maze using a nonmatch-to-sample paradigm showed impaired acquisition of the task by all lesioned groups. An open field maze showed no significant differences in horizontal activity between groups. These results suggest independent effects of the two lesions, and that NE depletion is able to reverse the cholinergic deficit in some tasks.

511.20

MOUSE STRAIN DIFFERENCES IN MORRIS WATER TASK PERFORMANCE. B.F. Petrie and N.M Standish. Department of Psychology, Red Deer College, Red Deer, Alberta, Canada, T4N 5H5.

Pigmented and Albino mice were swum for 30 days, to test for spatial learning ability, in the Morris water task. Albino animals were unable to reliably find the platform, while the time taken by Pigmented mice in escaping the pool declined rapidly, reaching asymptote within 7 days. Results are discussed in the context of genetic differences in the appropriate selection of strategy, that may have correlative anatomical and neurochemical underpinnings. Implications for memory augmentation and deficit are also analyzed.

512.1

INTERSPRCIES VARIATION IN THE VOLUME OF HVC, A SONG-RELATED AVIAN NUCLEUS. T.J.DeVoogd, J.R.Krebs* and S.D.Healy*. Dept. of Zoology, Oxford University, Oxford and Dept. Psychology, Cornell Univ., Ithaca, NY 14853

The avian song system is an interconnected group of brain nuclei that are believed to be responsible for song production in songbirds (Nottebohm et al., 1976). The telencephalic nucleus most directly implicated in song production is Hvc (higher vocal control). There is considerable variation in the volume of HVc in males, both within and between species. This variation is correlated with syllable repertoire size in canaries (Nottebohm et al., 1981), but not in red-winged blackbirds (Kirn et al., 1988). In two populations of marsh wrens, Hvc is larger in birds from the one with large syllable repertoires (Canady et al., 1984). These studies suggest that HVc volume is somehow related to song capacity but the nature of the relation is unclear. In the present study, the volumes of Hvc, the telencephalon and the parahippocampus were calculated for 26 species of songbird. In this sample, Hvc (L+R) varied in volume from .212 mm^3 (chiff chaff) to 5.728 mm^3 (magpie). Telencephalon and Hvc volume are positively correlated (r = .41). Telencephalon and parahippocampus volume are more closely related (r = .88). Thus, most of the variation in the volume of the parahippocampus can be accounted for by allometric relations; most of the variation in Hvc volume cannot. The residual Hvc volume was then compared to indices of species-typical song duration and variety (Hamilton and Zuk, 1982; Read and Weary, 1990) Preliminary analyses suggest that the remaining variation in Hvc volume is not related to song duration or the number of different syllables in a bird's repertoire, but is related to the number of different songs that a bird is able to produce.

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512.2

LOSS OF SPECIES-SPECIFIC SONG RESPONSES AFTER LESION OF A FOREBRAIN NUCLEUS IN FEMALE CANARIES. E. A.Brenowitz. Depts. of Psych. & Zool., Univ. of Washington, Seattle WA 98195.

Song production in male birds is regulated by a network of forebrain nuclei. Why do females that do not normally sing have these same nuclei, albeit in reduced form? One hypothesis is that these regions act in song perception, as shown for males (Margoliash '83; Williams '85). I tested this by examining the behavioral responses of female canaries to song before and after lesions of the caudal nucleus of the ventral hyperstriatum (HVc). Estradiol-implanted females were exposed to male canary song and to white-crowned sparrow song, and the number of copulatory solicitation displays performedwas recorded. No bird gave > 1 display to sparrow song. When females gave ≥ 5 displays to canary song, they received either sham or electrolytic lesions to HVc. I then tested post-lesion responses to canary and to sparrow song. When the caudal medial portion of HVc was destroyed, birds responded equally strongly to canary and sparrow song. Shams and lesioned birds with intact caudal medial HVc continued to respond only to canary song. Thus, lesions of caudal medial HVc seem to eliminate conspecific song recognition without disrupting song responsiveness, supporting the hypothesis that HVc acts in song perception in non-singing female birds. (Supported by NIH DC00487 & Sloan Fellowship)

512.3

BILATERAL LMAN LESIONS IN ADULT MALE CANARIES AFFECT SONG IN DIFFERENT WAYS WITH DIFFERENT LATENCIES. R.Suter, A.Tolles, M.Nottebohm and F.Nottebohm. Rockefeller Univ. Field Research Center, Millbrook, NY 12545.

The lateral magnocellular nucleus of the neostriatum (LMAN) was bilaterally lesioned in 6 adult (17-months old) male canaries in mid-September, when their song was very variable. The song of these birds was recorded during the following 6 months; the birds were then killed and their brains prepared for histology. Immediately after the lesion song became extremely stereotyped -- more so than in canaries in breeding condition -- successive repetitions of a same syllable being virtual carbon copies. However, within 2-3 weeks syllable diversity plummeted and birds started to produce uncommonly long tirades of just a few syllable types. These few syllable types were often delivered in a stammering fashion. We infer that in adult canaries in end-of-summer plastic song the recursive circuit to which LMAN belongs is necessary for song plasticity and for the acquisition, production and sequencing of complex learned repertoires.

512.4

DEAFENING AFFECTS CELL RECRUITMENT IN THE HVC OF ADULT FEMALE CANARIES. F.Nottebohm, B.Simpson and B.O'Loughlin.Rockefeller Univ. Field Research Center, Millbrook, NY 12545.

Six adult female canaries were deafened, 7 served as controls; 2 weeks later both groups got E2 implants and were placed facing cages with singing males. All females received 10 injections of 3H-thymidine (50uCi per inj. at 12h intervals) starting three days later. Their brains were processed for autoradiography six weeks after the last injection. The boundaries of the high vocal center (HVC) were defined using cresyl violet. The HVC of deaf and hearing birds did not differ in volume, number of neurons, glia and endothelial cells, area of overlying ventricular zone (VZ) and % of labeled neurons. However, the % of labeled VZ cells overlying HVC and the % of labeled HVC glia and endothelial cells were higher in the deaf than in the hearing birds (R+L sides combined). Interestingly, the effect on glia and endothelial cells was significant only on the right side. Mean nuclear diameters of labeled HVC neurons were larger on the left than on the right side of hearing birds. Deafness affected turnover and differentiation of HVC cells, particularly on the right side.

512.5

NEURONAL CLUSTERS: INCORPORATION SITES FOR NEURONS BORN IN ADULT CANARY FOREBRAIN. J. R. Kirn and F. Nottebohm, The Rockefeller University, Field Research Center, Tyrrel Road, Millbrook, NY 12545.

Neurons generated in the adult canary telencephalon are incorporated into vocal control nucleus HVC (Higher Vocal Center), where half or more become long-projection neurons within the efferent pathway controlling learned song (Alvarez-Buylla & Nottebohm Soc. Neurosci. Abst. 383.10, 1989; Kirn & Nottebohm, Soc. Neurosci. Abst. 383.9, 1989). Some new neurons also make direct soma-soma contacts with other neurons intrinsic to HVC, forming neuronal "clusters" (Burd & Nottebohm, JCN, 240:143-152, 1985). In the present work, we quantify this phenomenon and identify neuron types found in clusters. Each of 9 adult male canaries (17 month) received a total of 14 injections of ^{3}H-thymidine (6.7 Ci/mmole, 2.5 uCi/gm bwt., two injections/day over 7 days). Birds were sacrificed 30 days later and 6μ brain sections were processed for autoradiography and counterstained with cresyl violet. Roughly 40% of ^{3}H-labelled HVC neurons (x=38%; range=30-52%) were in clusters containing 2-4 unlabelled neurons. This value is probably an underestimate due to cell/cluster splitting during tissue sectioning. The projection patterns of clustered neurons were explored in additional male canaries (n=4) who received stereotaxic injections of the retrograde tracers Di-I or Fast Blue into Area X and Fluoro-gold into RA (Robustus Archistriatalis), the two targets for HVC projection neurons. A common cluster arrangement of a single Area X-projecting neuron, surrounded by 2-3 RA-projecting cells was observed. Thus, the major projection patterns of HVC are reflected in smaller neuronal assemblies. Neuron clustering is associated with synchronized activity in other systems (Theodosis et al., Nature 332: 738-40, 1986). In HVC, the activity of newly formed neurons may be constrained by that of preexisting neurons within clusters--a potential mechanism for the preservation and amplification of information in a system which undergoes turnover of some of its elements. It is also possible that newly formed neurons alter cluster response properties.

512.6

EFFECT OF UNILATERAL DENERVATION ON THE ACOUSTIC OUTPUT FROM EACH SIDE OF THE SYRINX IN SINGING MIMIC THRUSHES. R. A. Suthers and R. S. Hartley*. Sch. of Med. and Dept. of Biology, Indiana Univ., Bloomington, IN 47405.

Microbead thermistors implanted in each primary bronchus of adult male catbirds (Dumetella carolinensis) and brown thrashers (Toxostoma rufum) were used to record the acoustic contribution from each side of the syrinx before and after unilateral denervation of the syringeal muscles. Thermistors responded to air movement produced by the vibrating syringeal membranes. In these birds, paralysis of the muscles on either side of the syrinx resulted in an abnormal song, but the effect was usually slightly greater after the left side was paralyzed. Denervation of either the left or right side of the syrinx increased the number of syllables to which that side contributed sound. Few or no syllables were produced by the intact side alone and most post-operative syllables contained simultaneous contributions from both sides of the syrinx. The sound generated on the denervated side usually consisted of a fundamental with an abnormally low frequency and multiple harmonics. The frequency of this fundamental typically paralleled changes in the rate of airflow on that side of the syrinx, which in turn followed changes in the driving subsyringeal pressure. Sound from the intact side was essentially normal. The abnormal post-operative song in these birds is primarily due to their inability to regulate resistance or membrane tension on the operated side of the syrinx. We postulate that since the denervated side can no longer be silenced by adduction, its relaxed medial tympaniform membrane vibrates at a frequency determined by the rate of airflow across it. (Supported by grant BNS 87-20192 from N.S.F.).

512.7

GABAERGIC INHIBITION CONTRIBUTES TO NON-LINEAR SUMMATION AMONG MULTIPLE FREQUENCY CHANNELS IN THE BARN OWL'S INFERIOR COLLICULUS.

K. Mori[1*], I. Fujita[1,2] and M. Konishi[1].
[1]Div. of Biology, Caltech, Pasadena, CA 91125 and [2]Lab. for Neural Information Processing, RIKEN, Wako, Saitama 351-01 Japan.

Interaural time difference (ITD) is the primary azimuthal sound localization cue for the barn owl. Since ITD is processed in separate frequency channels in the brainstem pathway, neurons therein cannot distinguish ITD from interaural phase difference. Instead, they respond to multiple ITD's that give rise to the same phase difference ('phase ambiguity'). However, cells in the external nucleus and some cells in the lateral shell of the central nucleus (ICL type 2 cells of Fujita & Konishi, *Soc.Neurosci.Abstr.*, **15**:114, 1989) of the inferior colliculus solve the ambiguity by gathering inputs from different frequency channels. We investigated the interaction among frequency channels in those cells, and examined the role of $GABA_A$ inhibition in the process by iontophoretic application of its specific antagonist, bicuculline methiodide (BMI). Single units in the inferior colliculus of anesthetized barn owls were isolated with a multibarrel microelectrode. Two tones of different frequencies were presented binaurally. ITD vs. response curves for simultaneous presentation of two tones were compared with those for separate presentation of the same tones. Three types of interaction were noted; linear summation, non-linear facilitation, and non-linear suppression. Some cells changed type of interaction depending on the frequency-pair used. Iontophoretic application of 5mM BMI converted non-linear responses to a linear type to a varying degree. These results demonstrate the complex nature of the non-linear interaction across frequency channels in the inferior colliculus of the barn owl, as well as the involvement of GABAergic inhibition in this process. (Supported by the Del E. Webb Foundation, the Uehara Memorial Foundation, NIH, and the Japanese Ministry of Education, Science and Culture)

512.8

Focusing in Owls and Raptors Measured by Photorefraction

H. Howland, M. Howland & J. D. Pettigrew, Division of Biological Sciences, Cornell University, Ithaca, N.Y. ,14853 & Vision Touch and Hearing Research Centre, Department of Physiology, University of Queensland, St. Lucia, Qld. 4067

Previous work on a number of species of owls has shown that these birds exhibit a very great range of accommodative abilities which are roughly correlated with their size. Large owls exhibit small accommodative ranges while small owls generally exhibit large ones. An exception to this rule is the (medium sized) barn owl, Tyto alba, which showed a greater than 10 diopter accommodative range (1). We have initiated an investigation of the focusing abilities of Australian owls and raptors including three species of tytonid owls, the boobook, and a sparrow hawk, hobby falcon and black kite. We have employed both orthogonal photorefraction and photo-retinoscopy, using for the latter both infra red and visible light. We have found that the speed and range of accommodation of the raptors exceeds that of the Australian Tytonidae. We obtained a clear neutralizing photoretinoscopic refraction of the sparrowhawk (Accipiter cirrhocephalus) showing that it accommodated through 10 diopters. Particularly interesting was the fact that the peripheral fields of the hobby falcon (Falco longipennis) and the sparrow hawk exhibited considerable astigmatism. The implications of the focusing abilities of these birds and the optical qualities of their eyes for their visual capacities will be discussed. Facilites permitting, several of the optical refractive techniques will be demonstrated. Supported in part by NIH grant EY-02994 & a Fogarty fellowship to HCH and a grant from the ARC to JDP.

(1) Murphy, C. J. & Howland H.C. (1983) J. Comp. Physiol. 151:277-284

512.9

ACOUSTICALLY RESPONSIVE UNITS IN THALAMIC REGIONS AFFERENT TO THE AVIAN BASAL GANGLIA

S.E. Durand,*J.M. Tepper & M.-F. Cheng, Institute of Animal Behavior and Center for Molecular and Behavioral Neuroscience, Rutgers University, Newark, NJ 07102

Recently, Area X, a region within the oscine parolfactory lobe (LPO) that is a component of vocal pathways, has been implicated in song acquisition in vocal learners. In the Ring dove (*Streptopelia risoria*), a non-vocal learner, caudolateral LPO has not been demonstrated to exhibit a specialized subdivision associated with vocal pathways. However, in the dove, caudal LPO apparently receives auditory input from the thalamic nucleus ovoidalis (Ov). Additionally, a thalamic region, caudal to the thalamic projection of Area X in songbirds, contains acoustically responsive units and is retrogradely labeled from caudal LPO. The possibility that caudal LPO is a region of the basal ganglia involved in acoustic and motor integration, with reciprocal connections.to the thalamus, is a question deserving further investigation across avian species.

The projection from Ov to caudal LPO and the medial paleostriatum augmentatum (PA), initially revealed by the anterograde tracer *Phaseolus vulgaris* leucoagglutinin (PHA-L), was confirmed with the retrograde fluorescent dye, fluorogold. An injection encompassing caudal LPO and anteromedial PA also heavily labeled cells in the caudolateral region of the dorsomedial nucleus of the posterior thalamus (DMP), as well as in the area ventralis of Tsai. Injections that encompassed anterior LPO, but spared its caudal regions, failed to label neurons in Ov or DMP.

During our study of the acoustic responses of Ov neurons, 4 acoustically responsive units in the DMP/DIP border region were recorded in urethane anesthetized subjects. In the absence of acoustic stimulation, these units exhibited low levels of activity, ranging from 0.2 to 0.5 spikes/s and exhibited long latency responses (>25ms) to 500 ms noise bursts. Tone pulses of 200Hz elicited bursts of activity from 2 of these units; one also responded robustly to 500Hz tones. A more dorsally located unit showed only weak responsiveness to frequencies in the range of 2 kHz. A minor projection from Ov to caudal DMP was revealed previously with PHA-L, but it may not constitute the source of auditory input to these units.
Supported by Johnson and Johnson Discovery Grant and Charles and Johanna Busch Fund and MH-45268.

512.10

HIPPOCAMPAL LESIONS IMPAIR NAVIGATIONAL MAP DEVELOPMENT IN HOMING PIGEONS.

V. P. Bingman, P. Iaolé*, G. Casini* and P. Bagnoli. Dept. Psychology, Bowling Green State Univ., Bowling Green, OH 43403.

The homing pigeon navigational map allows birds to determine their position and take up an approximate homeward bearing from distant, unfamiliar locations. The navigational map shares many characteristics with the "cognitive map" found in the psychological literature, and may be the best example of a naturally occurring cognitive mapping system. The hippocampus has been found to play a critical role for spatial navigation in laboratory rodents. Surprisingly, previous research has shown that hippocampal ablation has no discernible effect on the functioning of the pigeon navigational map in adult birds with homing experience. In contrast, the present study demonstrates that hippocampal ablation in young pigeons who have yet to acquire a navigational map strikingly impairs their latter ability to take up a homeward bearing from distant unfamiliar locations. The data suggest that the avian hippocampus plays a critical role in navigational map acquisition while playing no further necessary role once the map is formed. Alternative explanations will be explored.

512.11

VISUAL FOREBRAIN AND EATING IN THE PIGEON.

R. Jager*, U. Schall*, J.J.Arends, R. W. Allan* and H. P. Zeigler. Biopsychology Program, Hunter College, (CUNY), New York, NY 10021.

The role of forebrain visual structures in the sensorimotor control of eating in the pigeon was examined using a technique combining unilateral hemispherectomy with monocular occlusion to produce a reversible "visual decerebration". Given the hodology of the visual system (complete decussation of the visual pathways to the forebrain) input to the eye contralateral to the intact hemisphere will access, unilaterally, all components of the tectofugal and thalamofugal pathways. For the eye contralateral to the ablated hemisphere, the forebrain components of these pathways will be eliminated. By alternately occluding the eye contralateral to the intact and ablated hemisphere, subjects could be tested first as visually "normal" and then as visually "decerebrate".

When tested in the Binocular condition or using the eye contralateral to the intact hemisphere, subjects showed no impairment on measures of ingestive efficiency, discrimination of food from non-food items, peck localization and the amplitude scaling of gape (interbeak distance) during grasping. When using the eye contralateral to the ablated hemisphere, there were significant impairments in ingestive efficiency, peck localization and discrimination of food from non-food items but no effects upon grasping and amplitude scaling behaviors. The dissociation of localization and grasping deficits suggests that these two tasks are mediated by different neural substrates. *Supported by NSF Grant BNS 88-10722 and NIMH Grant MH-08366.*

512.12

AUTORADIOGRAPHIC LOCALIZATION OF MUSCARINIC CHOLINERGIC RECEPTORS IN THE BUDGERIGAR BRAIN

G.F. Ball Dept. of Psychology, Boston College, Chestnut Hill, MA 02167

Budgerigars (Melopsittacus undulatus) a member of the parrot order, possess a neural vocal control system that is possibly homologous, at least in part, to the complex of nuclei that control vocal behavior in the songbird order. A comparison of the transmitters present in the song control nuclei in these two distantly related avian groups may address the question of homology and clarify whether vocal behavior is regulated in a similar fashion in these two taxa. I therefore used in vitro quantitative autoradiography to map the distribution of muscarinic cholinergic receptors, a receptor known to be present in several song control nuclei in songbirds, in male and female budgerigars. Muscarinic receptors were labelled using (^{3}H) N-Methyl scopolamine (1 nM conc. ± 5 µM atropine). Slices were exposed to tritium sensitive film for 1 week. Autoradiograms were analyzed using an image analysis sytem. The highest levels of specific binding were detected in parts of the basal ganglia such as the parolfactory lobe and the paleostriatum augmentatum. Moderate binding was detected in the hypothalamus at the level of the paraventricular nucleus. Of the song control nuclei, the nucleus intercollicular (ICo) in the mid-brain was specifically labelled. No structure could be discerned that resembled the robust nucleus of the archistriatum (RA) homologue. At the level of the anterior commissure a lateral structure exhibiting high specific binding was detected that was near the putative homologue of the caudal part of the ventral hyperstriatum (HVc) in the budgerigar. However, it did not appear to correspond precisely to this nucleus. These data suggest potential differences in the regulation of vocal behavior in parrots and songbirds.

513.1

EFFECT OF DIETARY FAT SOURCE ON MACRONUTRIENT SELECTION B. J. Mullen and R. J. Martin, Dept. of Foods and Nutrition, Dawson Hall, Univ. of Georgia, Athens, GA 30602

We have previously reported that type and level of dietary fat can influence an animal's subsequent preference for carbohydrate (CHO) and protein (PRO). Rats fed a 34% tallow diet show a subsequent preference for a high PRO diet while those fed 34% corn oil show a preference for a high CHO diet. The present study was undertaken to determine if the factor(s) responsible for enhancing PRO consumption in the tallow fed animals is restricted adipose tissue. Hence, we tested the effect of another beef fat, butter, which is derived from secreted lipid rather than a constituent of adipose tissue. Male, Sprague-Dawley rats (75-99 g) were divided into 3 groups and fed diets containing either 34% corn oil, tallow or butter for 2 days. These diets were then replaced with 2 diets given simultaneously to test dietary selection: 1) 15% CHO/60% PRO and 2) 65% CHO/ 10% PRO. Animals previously fed corn oil selected more CHO and less PRO than did animals fed either tallow or butter. The amounts of PRO and CHO selected by animals fed tallow or butter were not significantly different. These results suggest that the factor(s) responsible for altering diet selection are present in both adipose tissue and milk fat.

513.2

ADRENALECTOMY AND CORTICOSTERONE REPLACEMENT: EFFECTS ON MACRONUTRIENT INTAKE. D.L. Tempel, M. Yamamoto*, T. Kim* and S.F. Leibowitz. Rockefeller Univ. N.Y. 10021.

Adrenalectomy (ADX) decreases food intake and body weight and prevents the development of various forms of obesity. The present experiments studied macronutrient intake patterns in rats with "complete" ADX (blood corticosterone [CORT] levels < 2 µg%), "incomplete" ADX (blood CORT levels >3 µg%), and after low vs. high doses of CORT replacement.

Results indicate that, in complete ADX rats, both 24hr carbohydrate intake (-25%; $p<0.05$) and 24hr fat intake (-35%; $p<0.05$) are decreased, whereas in incomplete ADX rats, only fat intake is suppressed (-40%; $p<0.05$). This suggests that low circulating CORT levels are sufficient to maintain 24hr carbohydrate but not fat ingestion. This suppression of carbohydrate feeding occurs almost exclusively during the first 2 hrs of the dark cycle, when endogenous CORT levels as well as natural carbohydrate feeding normally peak. In contrast, the decrease in 2hr fat intake is only a small portion (20%) of the total 24hr fat suppression seen in both complete and incomplete ADX rats, which appears to occur uniformly throughout the feeding cycle.

Consistent with the finding that carbohydrate feeding in the early dark is lost only in complete ADX rats with <2 µg% CORT, tests with CORT replacement show that low levels of administered CORT (0.05-0.5 mg/kg via s.c. injection, implants or orally), which raise blood hormone levels to between 1 and 5 µg%, are effective in restoring early dark carbohydrate feeding; however, only higher doses of CORT (>2.0 mg/kg), resulting in blood levels of >10µg%, effectively restore 24hr fat intake. These results indicate that nutrient intake is differentially affected by ADX at different times of the feeding cycle and that low levels of CORT are sufficient to maintain or restore carbohydrate intake, while higher levels are necessary to restore fat intake. It is suggested that different steroid receptors in the brain may underlie these effects.

513.3

VARIATIONS IN THE EFFECTIVENESS OF TYPE II RECEPTOR STIMULATION ON FEEDING AND WEIGHT GAIN IN RATS. L. Devenport*, C. Hicks, and R. Stith. Dept. Psychol., Univ. Oklahoma, Norman OK 73019 and Dept. Physiol., Univ. Oklahoma Hlth. Sci. Ctr., Oklahoma City OK 73069.

Of the numerous metabolic functions of corticosterone (Cort), virtually all can be assigned to the message derived from its type I receptor. This opens the question as to the role of the high density/low affinity type II receptor. The endogenous type II signal is relatively brief and is delivered against a background of type I saturation. The present study examined the relative effectiveness of this pattern of signalization. The type II agonists, dexamethasone (Dex) and RU28362 (RU) were chronically administered in the presence or absence of continuous type I stimulation. Dex and RU were delivered by continuous infusion or by single daily injections at 7 pm. Administered alone, RU and Dex reduced body weight gain, feeding efficiency, and food intake. Continuous infusion was considerably more suppressive than injection and produced wide fluctuations in daily body weight gain. The suppressive effects of Dex and (especially) RU were attenuated when type I receptors were occupied; the lowest dose of RU (5ug/kg/day) slightly augmented the strong anabolic effect of the type I agonist (Aldo). The outcome of type II receptor stimulation depends upon mode of administration and concurrent type I activity.
Supported by DK 34347 to L.D.

513.4

BODY WEIGHT GAIN IN RATS AFTER FOOD RESTRICTION AND ADRENALECTOMY (ADX) IS INCREASED BY INTRAVENTRICULAR (IVT) CORTICOSTERONE (CORT). P.K.Green, C.W.Wilkinson, S.C.Woods. Depts Psych and Psychiat and Behav Sci, Univ Wash, Seattle WA 98195 and VA Med Ctr, Tacoma WA 98493.

ADX gold thioglucose-treated mice reportedly increase food intake and body weight after a single IVT injection of CORT. To study the role of CORT on weight gain, Long-Evans rats were either allowed free access to chow (AD LIB group) or food restricted (RES) to half baseline daily food intake (18 g/day) for two weeks prior to ADX or sham operation. The next day rats received a single IVT injection of 100ug CORT in 2 ul propylene glycol vehicle, or 2 ul vehicle (VEH). This dose of CORT had no effect on body weight gain when administered subcutaneously. In both AD LIB and RES groups, ADX arrested or decreased weight gain over the six days following the injection (AD LIB: mean loss of 1.8g for ADX vs. gain of 14.9g for SHAM; RES: ADX gained 46.6g, SHAM 74.3g). In the AD LIB group, IVT CORT non-significantly reduced weight gain relative to their controls. In the RES group, IVT CORT significantly decreased body weight gain in SHAM rats (CORT: 68.4±7,4g, VEH: 82.6±4.4g), yet significantly increased body weight gain in ADX rats (CORT: 59.7 ±5.6g, VEH: 36.7±7.5g). It is concluded that centrally-acting CORT has a role in determining body weight regain after food restriction in non-obese rats.

513.5

RATS OVERFED AS NEONATES BECOME OBESE AND INSULIN RESISTANT AS ADULTS. J. Diaz, E. McGarvey, G. Watkins* and A. Scheurink*. Dept. of Psychology, University of Washington, Seattle, WA 98195

Animal models of the various aspects of non-insulin-dependent diabetes (NIDDM) have typically used mutant rat strains and or severe pharmacological insults such as streptozotocin lesions of the pancreas. This experiment precipitated aspects of NIDDM with environmental manipulations during development.

At four days rat pups were randomly assigned to one of three groups 1) mother reared in litters of ten (MR) 2) gastrostomy-fed for nine days (Days 5-14) to match the growth of the MR group (WM), and 3) gastrostomy-fed with excess formula to accelerate growth (OF). At Day 14, the gastrostomy-fed groups were returned to dams. All animals were weaned on Day 21. Females were bred at age 65 days. At 180 days females who had a successful pregnancy received IV glucose challenges via chronic jugular catheters.

Overfed animals became frankly obese after puberty. Only the less severely obese females were able to carry a pregnancy to term, and their pups were significantly smaller than the offspring of sibling controls. An exaggerated insulin response to the IV glucose challenge was seen.

These data demonstrate that nutritional manipulations early in life may produce an adult with some of the characteristics of NIDDM.

513.6

EFFECTS OF LESIONING THE AMYGDALA, PARABRACHIAL NUCLEUS AND THE HYPOTHALAMIC PARAVENTRICULAR NUCLEUS ON LIPOPRIVIC AND GLUCOPRIVIC FEEDING. N.Y. Calingasan, B.W. Hutton* and S. Ritter. Department of VCAPP, Washington State University, Pullman, WA 99164-6520.

Previous results have shown that feeding, stimulated by mercaptoacetate-induced blockade of fatty acid oxidation (lipoprivic feeding), is dependent on vagal sensory neurons innervating the abdominal viscera. Feeding elicited by 2-deoxy-D-glucose-induced blockade of glucose utilization (glucoprivic feeding) does not require peripheral vagal neurons, but is abolished by area postrema (AP)/NTS lesion. In this study, lesions were placed in the central nucleus of the amygdala, parabrachial nucleus and the hypothalamic paraventricular nucleus, areas known to receive visceral sensory projections from the AP/NTS region, in order to identify central neural substrates for these controls of feeding. After recovery from surgery, rats were placed on a medium fat diet for at least 2 wks and then tested for lipoprivic and glucoprivic feeding. Electrolytic lesions which included the central nucleus of the amygdala abolished lipoprivic, but not glucoprivic, feeding. Kainic acid lesions of the lateral parabrachial area did not impair either control. Relatively large hypothalamic lesions destroying the paraventricular nucleus disrupted both controls. Thus, areas containing projections from the AP/NTS region may be important for glucoprivic and lipoprivic feeding. The projections critical for each control may be distinct, although convergent at some sites.
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513.7

ABLATION OF GLUTAMATE-SENSITIVE AREA POSTREMA NEURONS DOES NOT ABOLISH GLUCOPRIVIC FEEDING. M.G. Hulsey, D.K. Hartle and R.J. Martin. Depts. of Foods & Nutrition and Pharmacology. Univ. of Ga., Athens, Ga.

High plasma levels of monosodium glutamate (MSG, s.c.) cause a selective loss of glutamate-sensitive neurons (GSN) in the area postrema (AP). The neurochemical profile of most GSN in AP are tyrosine hydroxylase (TH) immunoreactive (IR) ① and/or 5HT IR ② (Phelix and Hartle, BRES ① in press, ② submitted for publication). Approximately 2500 neurons are killed in the AP while no pyknotic nuclei appear in the adjacent NTS or other circumventricular organ regions. Only subfractions of the total TH-IR and 5HT-IR AP cell populations are GSN. Remainder populations are glutamate-insensitive, even with a subsequent MSG treatment. AP/NTS has been implicated in the glucoprivic feeding response subsequent to 2-Deoxyglucose (2-DG) administration. We tested whether the GSN of AP are critical to this response. We treated eight 220 g male SD rats with 6 mg/g s.c. MSG. Five days later, 4 of the rats were given a glucoprivic challenge. In the 3rd h of the 12 h photoperiod, 400 mg/kg 2-DG or saline was administered i.p. in a randomized block design (N=4 each group). Intake of ground chow was computer-monitored at resolutions of .5 Hz and .01 g. An analogous second trial was performed using an MSG dose of 9 mg/g. Cumulative food intake values for the 3h post-injection period were subjected to 1-way ANOVA. In both MSG treatments, ablation of the GSN in AP failed to inhibit the glucoprivic feeding response to 2-DG ($p < .008$). We conclude the GSN of the AP are not involved in the glucoprivic feeding response. Support: HLBI 37705.

513.8

THE EFFECT OF INTRAVENOUS INSULIN ON CEREBROSPINAL FLUID (CSF) BIOGENIC AMINE CONCENTRATIONS IN ANESTHETIZED RATS. **Thomas W. Castonguay and Wayne J. Kuenzel University of Maryland, College Park, Maryland 20742**

Intact rats will self-administer insulin i.v. if the concentration of each injection is within 50-100 mIU. The purpose of the present experiment was to measure the effects of insulin on changes in serotonin and dopamine metabolite concentrations in CSF. Adult rats were anesthetized, fitted with jugular vein cannulae, and injected with 1 of 3 doses of insulin: 100, 200 or 300 mIU. CSF was collected at 0, 10 and 30 min. after injection. Analysis of CSF samples was performed using HPLC with electrochemical detection. Within 30 minutes 5-HIAA increased 13, 31 and 22 percent over baseline levels in the 100, 200 and 300 mIU groups respectively. CSF DOPAC increased 50% in response to 100 mIU insulin within 10 minutes of injection. Higher insulin concentrations resulted in an inverse relationship with DOPAC, with a 22% decrease observed at 30 min. These results suggest that insulin favors increased serotonin and dopamine metabolism. (Supported in part by a grant from the Whitehall Foundation).

513.9

HYPERINSULINEMIA AND REDUCED SODIUM EXCRETION IN DIETARY OBESE, WEIGHT-CYCLED RATS. R. J. Contreras. Department of Psychology, University of Alabama, Birmingham, AL 35294.

The aims of the present study were to: (1) replicate the findings of Ernsberger and Nelson (*Am. J. Physiol.*, 254: R47-R55, 1988) reporting the development of mild hypertension in dietary obese Sprague-Dawley (S-D) rats exposed to 4 cycles of food restriction-refeeding (weight cycling) on a sweet milk diet; and (2) determine whether the mild hypertension was associated with changes in sodium excretion, hyperinsulinemia, pressor responsiveness to angiotensin II, and heart rate response to pharmacological blockade of the autonomic nervous system. Twenty-five male S-D rats were divided among 3 groups: 8 rats were fed Agway pelleted chow (Pellet); 8 rats were fed pelleted chow plus sweetened condensed milk (Milk); and 9 rats were exposed to 4 cycles of a 4-d fast alternated with 2-wk of refeeding pelleted chow and sweetened condensed milk (Cycled).

Dietary obesity and weight cycling resulted in significant elevations in body weight and terminal brown fat pad and white fad pad weights, hyperinsulinemia, and reduced sodium excretion. In contrast to Ernsberger & Nelson, weight cycling superimposed on dietary obesity did not alter (1) blood pressure, or (2) heart responses to sympathetic blockade with metoprolol and parasympathetic blockade with methylscopolamine. The pressor responses to intravenous administration of angiotensin were also unaffected by obesity and weight cycling. The dietary obese, weight-cycled S-D rat may not be a good animal model for human obesity-related hypertension.

Supported by NIH Grant HL-38630.

513.10

WHEN MAINTAINED ON A RESTRICTED DIET, RATS REARED IN ENRICHED ENVIRONMENTS DEFEND BODY WEIGHT BETTER THAN RATS REARED IN ISOLATION. **S.C. Fowler, J.M. Chase, M.J. Kallman, and P. Hopkins*. Depts. of Psychol. and Pharm., Univ. of Mississippi, University, MS 38677.**

Between the ages of 22 and 82 days rats were reared, under ad libitum food and water conditions, in either enriched environments (n=37) or in individual isolation cages (n=32). Then all rats were restricted to daily 1 hr feeding for the next 78 days. At this time enriched rats were significantly heavier than the isolates, $t(67)=3.275$, $p=0.002$. In addition, the difference between pre- and post-feeding body weights significantly favored the enriched rats by 4.63g, $t(67)=2.822$, $p=0.006$. These results suggest that superior defense of body weight by the enriched rats is behaviorally mediated via the enriched rats eating at a higher rate than the isolates. A second study replicated the first and further showed that (1) isolates are heavier than enriched rats when fed ad libitum and (2) limiting feeding to 1 hr/day does not abolish the brain-weight-increasing effects of enriched environment rearing. **Supported by DA 05310.**

513.11

DIET-INDUCED OBESITY (DIO) AND HIGH ENERGY DIET (HED) ADVERSELY AFFECT LOCAL CEREBRAL GLUCOSE METABOLISM (LCGU). B.E. Levin. Neurology Svc., VA Med. Ctr., E. Orange, NJ 07019, Dept. Neurosciences, NJ Med. Sch., Newark, NJ 07103.

Previous studies (Levin & Sullivan, 1989) showed that rats prone to develop DIO had defective activation of autonomic brain areas to food-related cues. Here, 24 adult male SD rats were fed an HED for 3mo; half became DIO, gaining 58% more weight, while the rest were diet resistant (DR), with the same weight gain as 12 chow-fed rats. Fasting rats were then trained to drink 1ml of 50% glucose in 1min associated with a tone cue. LCGU was then assessed in 20 brain areas, using [14C] 2-deoxyglucose (Sokoloff et al., 1977), with tone alone or with tone plus 0.15% saccharin in place of glucose. DR but not DIO rats increased LCGU 14% to saccharin intake in the n. tr. solitarius while overall LCGU was 14% lower in the central amygdalar n. of DIO rats. HED intake led to reduced inferior olive LCGU to saccharin and a 15% overall increase in medial amygdalar n. LCGU in DR and DIO vs. chow-fed rats. Thus, both DIO and HED intake adversely affect activation of brain autonomic areas to food related cues. This may play a role in development and perpetuation of DIO.

513.12

ESTRADIOL INCREASES SYMPATHETIC NERVOUS SYSTEM ACTIVITY IN RETROPERITONEAL ADIPOSE TISSUE. S.J. Lazzarini and G.N. Wade. Neuroscience and Behavior Program and Dept. of Psychology, Univ. of Massachusetts, Amherst MA 01003.

Estrogens have both central and peripheral effects on body weight in rats. Ovariectomy (OVX) results in body weight gain (primarily by increasing fat stores) and estradiol replacement therapy reverses these effects. The sympathetic nerves play a role in estradiol-induced fat pad weight losses in OVX rats. Denervation of retroperitoneal adipose tissue (RWAT) attenuates fat pad weight loss. This effect is not due to alterations in the concentration of cytosol estrogen receptors. We determined whether estradiol-induced fat pad weight losses are accompanied by increased RWAT norepinephrine (NE) turnover, an index of sympathetic activity. Rats were OVX and treated with estradiol benzoate (EB) or sesame oil vehicle. NE turnover was assessed by measuring the decline of tissue NE over time after injection of α-methyl-*p*-tyrosine, an inhibitor of tyrosine hydroxylase activity and thus NE biosynthesis. NE turnover in RWAT, but not heart, was significantly greater in animals treated with EB, suggesting that estradiol decreases fat pad weight in part by increasing sympathetic nervous system activity. It is possible that estradiol acts in the brain to regulate the activity of the sympathetic nerves. (Supported by NS 10873, DK 32976, and MH 00321.)

513.13

(-)*threo*-CHLOROCITRIC ACID DECREASES SHAM FEEDING OF SUCROSE IN RATS. S.C. Weatherford, W.B. Laughton, J. Salabarria* and D. Nelson*. Neurobiology and Obesity, Hoffmann-La Roche Inc., Nutley, NJ 07110.

It has been proposed that the novel anorectic agent, (-)-*threo*-chlorocitric acid (CA), decreases food intake through a reduction in gastric emptying (Pharm. Bio. Behav., 1981). If so, CA should not decrease sham feeding, a preparation where postingestive cues such as gastric distension are greatly minimized. Here we examine the effect of CA in rats sham feeding 20% sucrose relative to the appropriate "real" feeding condition.

RESULTS: Values are mean ± SEM % of baseline intake

	Real Feeding			Sham Feeding		
	Minutes of Feeding Tests					
Dose	0-15	0-60	0-120	0-15	0-60	0-120
100	75±5*	86±6	103±6	94±1	91±2	83±2
200	83±6	78±5*	92±6	105±3	84±2*	64±2*
400	52±9*	50±8*	42±6*	89±4	73±6*	49±7*

Tests were conducted in the a.m. after a 17 hr fast, N=8, doses are mg/kg, p.o. given 30 min before sucrose, * $p < .05$.

CONCLUSIONS: Since CA decreased sham feeding, its anorectic effect cannot be solely attributed to inhibition of gastric emptying. However, since CA was more potent in the real feeding condition relative to sham feeding and the time course of the response in the two feeding conditions was different, this suggests that part of CA's anorectic effect does depend on postingestive cues.

513.14

DUODENAL GLUCOSE INFUSION IN THE RABBIT. P.J. Geiselman, A. Acevedo-Cruz*, and D.S. Gray. Dept. Psychol., UCLA; Div. Diab., USC Sch. Med., Los Angeles, CA 90024.

Rapid duodenal glucose infusion (3 ml/min) increased chow intake in free-fed and meal-fed rabbits. Slow duodenal glucose infusion (1 ml/min) increased food intake in meal-fed rabbits but decreased food intake in free-fed rabbits, suggesting that daily pattern of meal-taking can determine whether glucose produces satiety or hunger.

To define further conditions under which glucose produces satiety or hunger, blood glucose and insulin were measured in free-fed rabbits. After fast duodenal glucose infusion, insulin increased sharply, followed by a precipitous decline in blood glucose. Slow duodenal glucose infusion increased insulin moderately, which failed to decrease glycemic levels significantly. Tests are being conducted to determine whether feeding responses vary across trials in free-fed rabbits (as found in meal-fed rabbits) and, if so, if changes in food intake are related to blood glucose and insulin responses.

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513.15

CONDITIONED SATIETY DEPENDS ON GASTRIC AND POSTGASTRIC SIGNALS. J. Miesner*, G.P. Smith, J.D. Davis, and P. Olcese*, New York Hospital-Cornell Medical Center, White Plains, NY 10605 and University of Illinois, Chicago, IL 60680.

When rats are given consecutive sham feeding (SF) tests, intake increases progressively to a maximum in 4-5 tests. Interspersing 2 real feeding (RF) tests between SF tests prevents this progressive increase in intake (Davis & Smith, 1990). This result suggests that a learned association between the flavor of the test diet and its postingestional consequences under real feeding conditions inhibits sham intake (conditioned satiety). To determine if the postingestional factor is of gastric and/or postgastric origin, 10 rats equipped with a surgically implanted, inflatable, pyloric cuff and a gastric fistula were tested with a 0.8M sucrose diet on 6 cycles of 2 RF tests followed by 1 SF test. On all tests the pyloric cuff was inflated to prevent postgastric stimulation by the ingested fluid. Under these conditions sham intake increased with experience ($p<.01$), but not as much as when no RF tests intervened. Since no progressive increase in sham or real intake occurred when food emptied normally from the stomach during real feeding, we conclude that postgastric stimulation is a necessary component of postingestional stimulation for normal conditioned satiety. The results also reveal a significant effect of gastric stimulation because the increase in sham intake observed when the ingested food accumulated in the cuff-closed stomach during RF tests was significantly less than the progressive increase in sham intake when sequential SF tests were administered without interspersing 2 RF tests.

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513.16

RUNNING WHEEL ACCESS AND EATING BEHAVIOR OF MALE RATS. R. Eikelboom. Dept. of Psychology, Wilfrid Laurier Univ., Waterloo, Ont N2L 3C5

Food consumption drops by about 30% when running wheels are made available (Looy & Eikelboom, Physiol Behav., 45:405, 1989). This study recorded only total daily food consumption and running. The nature of the change in feeding needs to be determined.

In the present study 8 male Sprague-Dawley rats (310-325g) where housed in individual cages (as weight controls n=4) or in special cages with pellet dispensers (45mg Rodent Chow Bio-Serv) providing ad lib food. For 9 days food and water consumption patterns were recorded in 5 sec bins. Then wheels were made available for 30 days and all three behaviors recorded. Wheels were closed and food and water consumption recorded for a further 30 days.

When rats had wheel access food consumption dropped by about 40%. By 30 days it had returned to about 80% of baseline. These changes were due to changes in meal number (from 18.8 to 10.7 meals a night when the wheel was opened to 13.6 after 30 days). There was no simultaneous change in the meal size. (Work sponsored by NSERC)

513.17

DAILY CYCLES IN SOME THERMOREGULATORY RESPONSES OF PIGEONS DURING FOOD DEPRIVATION AND RESTRICTED FEEDING.

D.L. Phillips*, M.E. Rashotte, and R.P. Henderson*. Psychobiology/Neuroscience Program, Department of Psychology R-54, Florida State University, Tallahassee, FL 32306-1051.

Core body temperature (Tb), whole-body thermal conductance (C), and whole-body heat loss (HL) were studied in pigeons during two conditions: **food deprivation** for several days, and **single daily feeding** to maintain body weight at 80% of ad libitum values for several months. Twenty-four hour measurements during the 12h:12h L:D cycle show: i) during **food deprivation**, C is reduced more rapidly than Tb; and, Tb develops a larger-amplitude cycle while the amplitude of the C cycle is reduced; ii) during **single daily feeding**, occasional variation in the amount of food presented markedly affects the amplitude of the Tb cycle, but not the C cycle; and, the time at which food is presented in the day alters the cyclic pattern of Tb and C, but does not affect total daily HL. In both conditions, C contributed more to the reduced HL than did Tb, indicating its predominance as an energy conservation mechanism.
(Supported in part by NSF grant BNS-8819941)

514.1

GABA$_A$ AND BENZODIAZEPINE BINDING IN ADULT AND PERINATAL RODENT SUPRACHIASMATIC NUCLEUS. M. Li* and J. L. Fuchs. Univ. of North Texas, Dept. Biological Sciences, Denton, TX 76203.

GABA$_A$ and associated benzodiazepine binding sites have been implicated in the control of rodent circadian rhythms. Certain GABA$_A$ and benzodiazepine agonists or antagonists can produce or block phase shifts, or alter the free-running period. Interest in these systems led us to examine [^{3}H]muscimol (GABA$_A$) and [^{3}H]flunitrazepam (benzodiazepine) binding in rat and hamster suprachiasmatic nucleus (SCN), the primary circadian pacemaker. Receptor autoradiography was used to test for the presence of rhythms which might underlie the phase-dependency of the pharmacological effects. No diurnal differences in ligand binding were found, using 6 rats at each of 8 time points in LD 12:12. In addition, there were no differences in light versus dark at night.

[^{3}H]Muscimol and [^{3}H]flunitrazepam binding increased in the rat SCN from embryonic day 18 to near adult levels by E20. The increases probably do not depend upon synaptogenesis, as very few synapses are present in the fetal SCN. The rises occurred around E19, when the SCN [^{14}C]2-deoxyglucose rhythm is first entrained to the maternal rhythm, suggesting that GABA$_A$ and associated benzodiazepine binding sites might participate in fetal entrainment. Supported by MH41865.

514.2

ALLOXAZINE BLOCKS THE HYPNOTIC EFFECT OF TRIAZOLAM IN RATS. **S.D.O'Connor and M. Radulovacki, Dept. of Pharmacology, Univ. of IL., Chicago, IL 60612.**

Triazolam, a benzodiazapine (BDZ) hypnotic, appears to mediate several of its effects through receptors coupled to the GABAergic system. It has been hypothesized that central effects of BDZs are mediated in part through the adenosine (ADO) system (Phillis and O'Regan, TIPS 9: 5, 1988). Recent work from our lab has demonstrated the interaction between these two systems, since chronic i.c.v. administration of a specific ADO transport inhibitor, soluflazine, mimicked the effect of chronic administration of triazolam, by decreasing radioligand binding to ADO A2 receptors in the striatum. To further examine the role of ADO in the hypnotic effect of BDZs, we measured the effect of acute administration of an ADO A2 receptor antagonist, alloxazine (ALX) on sleep induced by triazolam. Sprague-Dawley rats given 0.1 mg/kg triazolam i.p. showed decreased waking (25%) and increased total sleep (10%) as compared to controls during a 6 hour recording period ($p<0.05$). Pre-treatment of rats with 5mg/kg ALX i.p., which had no effect on waking by itself, resulted in a return to control levels of sleep when given in combination with triazolam. These results indicate that at low doses, sleep induced by triazolam may be a function of increased ADO activity that can be blocked by ADO A2 receptor antagonists.

514.3

DOES THE PHASE-SHIFTING EFFECT OF AN ACUTE INCREASE IN ACTIVITY ON CIRCADIAN RHYTHMS OF HAMSTERS INVOLVE BENZODIAZEPINE RECEPTORS? C. Wickland and F. W. Turek, Dept. Neurobiol. & Physiol., Northwestern U., Evanston, IL.

Treatment of golden hamsters with the short-acting benzodiazepine, triazolam, is associated with acute increases in activity and phase-dependent shifts in the circadian rhythm of locomotor activity which can be prevented by prior treatment with the benzodiazepine antagonist, RO-15-1788. Since transfer to a new cage with a running wheel a few hours before the onset of activity also induces phase advances in the activity rhythm in hamsters normally housed without wheels, we conducted this study to examine the effects of administration of RO-15-1788 on the phase-shifting effect of this transfer. Hamsters housed without running wheels in constant light were treated 4 hours before activity onset with (1) transfer to a new cage with a wheel for 1 hour (RO+Wh), (2) 5 mg RO-15-1788 and transfer to a new cage with a wheel for 1 hour, or (3) 5 mg RO-15-1788 alone (RO). Phase advances induced by RO+Wh ($\bar{x}$=46±22 min.) were smaller than those induced by Wh ($\bar{x}$=132±48 min.) and larger than those induced by RO ($\bar{x}$=15±8 min.), but were not significantly different from either. The number of wheel revolutions in group RO+Wh was significantly less than those in group Wh ($p<.01$). Also, the increase in total locomotor activity during the hour of treatment for group RO+Wh was significantly less than the activity increase for group Wh ($p<.05$), and significantly greater than the activity increase for group RO ($p<.01$). These results suggest that treatment with RO-15-1788 attenuates the phase-shifting effect of an acute increase in activity on the circadian rhythm of activity in hamsters. These results also indicate that RO-15-1788 has an inhibitory effect on the amount of increased activity that normally occurs after transfer to a new cage with a running wheel.

514.4

NOCTURNAL ADMINISTRATION OF FENTANYL: THE POTENTIATION OF THE THERAPEUTIC EFFECT IN RATS. R.A.Del Vecchio*, A.V.Wing*, C.A.Taylor, C.Tsai and M.J.Benvenga. Anaquest/BOC Health Care, Murray Hill, New Jersey, 07974.

Modern chronopharmacology involves the study of the effect of biological timing on the affinity and response of an organism to chemical agents. The present study investigated the behavior following nocturnal (2000-2400 hrs) or diurnal (0900-1400 hrs) administration of fentanyl to rats.

Male rats (N=6/dose) were treated with fentanyl intravenously and assessed for loss of righting (LOR). The ED50 for nocturnal administration (0.005 mg/kg) was approximately 3 times more potent than when fentanyl was administered diurnally (ED50 = 0.0175 mg/kg). In addition, the duration of LOR at night (2.68 min) was over 3 times shorter than when given during the day (8.76 min). The recovery index, assessed with a rotarod, indicated that the nocturnal recovery (31.3) was faster than the recovery during the day (18.2). Preliminary biochemical analysis (night vs. day) has revealed a trend difference in kappa binding while mu and delta binding remains to be reconciled. These results may have implications in the clinical use of opiates.

514.5

EFFECTS OF MUSCARINIC ANTAGONISTS ON SLEEP. R.K. Zoltoski, J. Velazquez-Moctezuma, M. Shalauta*, S.L. Lucero*, C. Floyd*, J.C. Gillin, P.J. Shiromani. Dept. of Psychiatry, San Diego VAMC, and Univ. of CA, San Diego, San Diego, CA 92093.

It is well known that muscarinic receptors regulate the onset of REM sleep. Recent findings from our laboratory indicate that REM sleep in cats can be elicited by M2 muscarinic receptor stimulation in the medial pontine reticular formation. The present study was designed in rats to assess the participation of M1 receptors in the regulation of REM sleep. Sprague-Dawley rats that were chronically instrumented for sleep recordings were injected in a randomized fashion with five doses (0,0.5,1,2, and 4 mg/kg) of trihexyphenidyl, a selective M1 antagonist or scopolamine, a non-selective muscarinic antagonist. There was no significant change in sleep latency by either drug. Following all doses of scopolamine, a significant increase in REM latency, as defined from time of sleep onset, was observed. However, a significant increase in REM latency was observed following only the highest two doses of trihexyphenidyl. Additionally, the preliminary effects of biperiden (2,4, and 8 mg) in human subjects suggest that M1 receptors exert no influence on the regulation of REM sleep. These preliminary findings in conjunction with our cat data support our hypothesis that M1 receptors do not play a significant role in the onset of REM sleep.

514.6

OLFACTORY BULBECTOMY LENGTHENS TAU IN MALE SYRIAN HAMSTERS David R. Pieper, Melinda Thompson* and Catherine Lobocki*, Providence Hospital, Department of Physiology, Southfield, MI 48037

Olfactory bulbectomy (BX) increases gonadotropin secretion in male hamsters. BX also lengthens the free-running period of locomotor activity (tau) in rats and mice. It is possible that a longer tau could be causally related to the increase in gonadotropin release. The present study examined whether BX lengthens tau in hamsters.

Twenty-three day old hamsters were BX or sham BX (SH) and one half of each surgical group was placed in cages with exercise wheels (EX group) while the other half was housed in similar cages without wheels (SED). At this time all animals were on a 14L:10D photoperiod. Eight weeks later, all animals were transferred to an animal room on constant darkness (DD) in order to assess tau. Activity rhythms were monitored with an Esterline-Angus recorder.

While the animals were still on 14L:10D, BX delayed activity onset in relation to lights off from .15 ± .034 hr to .37 ± .025 hr ($p < 0.001$). When the animals were placed on DD, the mean tau was 24.08 ± 0.04 hr in the SH animals compared to 24.41 ± 0.03 hr in the BX group ($p < 0.001$).

In conclusion, the olfactory bulbs exert a tonic influence on the circadian clock of hamsters so that the period of the clock is longer. It remains to be determined by what mechanism or neural pathway this influence is mediated, and whether the longer tau is involved in the effect of BX to increase gonadotropin secretion.

514.7

INTRACELLULAR RECORDINGS FROM TUBEROMAMMILLARY NEURONES IN AN ISOLATED AND PERFUSED WHOLE BRAIN OF GUINEA-PIG IN VITRO.

A. KHATEB, M. SERAFIN AND M. MUHLETHALER, *Dept de Physiologie, CMU,1211 Genève 4, Switzerland*

Following extracellular recordings in behaving cats it has been suggested that histaminergic neurones from the tuberomammillary nucleus (TM), which have widespread projections in the brain, play an important rôle in the control of arousal (Lin et al., Brain Res., 1989, 479: 225-240). Recently rat TM neurones were shown to survive well in vitro in an explant of the basal hypothalamus (Haas and Reiner, J.Physiol., 1988, 399: 633-646) and to resemble in many respects other aminergic neurones. While this technique is useful for assessing the intrinsic properties of TM neurones (Green et al., J.Physiol., 1990, 420:149-163), it cannot resolve their synaptology, which is important in order to understand their rôle in the control of behavioral states. It was thus of interest to determine whether such TM neurones could survive in an isolated and perfused whole brain (IWB) of guinea-pig in vitro and in a first step to determine whether their properties would resemble those of TM neurones recorded in the rat explant. We recorded from 20 TM neurones in the IWB. As shown previously TM neurones were mainly characterized by their broad action potential and the presence of a transient rectification due to an A current. Their basic properties were (mean ± SEM): resting potential, -58.3 ± 1.23 mV (n=11); spike amplitude: 64.4 ± 2.04 mv (n=14); spike width 2.16 ± 0.079 ms (n=13). These neurones discharged spontaneously at a rate of 13.95 ± 1.76 spikes/s (n=11). It is concluded that TM cells survive well in the IWB and that this model can be usefully applied to their further study in vitro (supported by a Swiss NSF grant no. 31-26495.89)

514.8

NEUROTRANSMITTER MODULATION OF FIRING MODE IN THALAMIC INTRALAMINAR NEURONS AND ITS FUNCTIONAL IMPLICATIONS Anne Williamson and David A. McCormick Neuroanatomy Section, Yale Medical School.

The thalamic intralaminar nuclei collectively innervate wide regions of the neocortex and have been proposed to contol in part the excitability and pattern of activity generated in thalamocortical circuits. Indeed, neuronal activity in the intralaminar thalamic nuclei exhibits two distinct states: rhythmic burst firing during drowsiness and slow wave sleep, and single spike activity during periods of waking, attentiveness and REM sleep. The ascending modulatory neurotransmitter systems which release acetylcholine (ACh), histamine (HA), norepinephrine (NE) or serotonin (5-HT) determine which state these neurons exhibit. Here we investigate, using standard extracellular and intracellular electrophysiological techniques, the actions of these four neurotransmitters on thalamocortical relay neurons in the guinea pig centromedian and reuniens nuclei, maintained as thalamic slices *in vitro* .

Extracellular recordings revealed that at rest intralaminar neurons fire spontaneous high frequency (200-400 Hz) bursts of action potentials at a regular rate of 0.5-3.0 Hz. Local application of HA, NE, or ACh resulted in an abolition of rhythmic burst firing and the promotion of single spike activity. In contrast, application of 5-HT resulted in an abolition of rhythmic burst firing without the promotion of single spike activity. Intracellular recordings reveal that these changes in firing mode are mediated by three ionic actions: 1) a slow depolarization associated with a decrease in membrane conductance; 2) a decrease in response to large hyerpolarizing current pulses, possibly mediated by an enhancement of the hyperpolarization-activated cation current I_h; 3) an abolition of the slow Ca^{++}-activated K^+ current I_{AHP} in a subpopulation of neurons. Together, these ionic actions potently inhibit the generation of rhythmic oscillations and promote single spike activity by depolarizing the neuron out of the range in which slow oscillations can occur, by reducing responsiveness to large inhibitory inputs, and by abolishing the slow afterhyerpolarization and consequently, spike frequency adaptation. These changes in the firing mode of intralaminar neurons may then prepare the cortex to receive information arising from the primary sensory nuclei.

514.9

STUDY OF BEHAVIORALLY DEPENDENT PAIRED-PULSE FACILITATION AND INHIBITION IN THE HIPPOCAMPAL CA1 REGION. F. Cao and L.S. Leung. Depts. of Clin. Neurol. Sci. and Physiology, Univ. Western Ontario, London, Ontario, Canada, N6A 5A5.

It has previously been reported that excitatory postsynaptic potentials and population spike evoked by stimulatum of afferents to the basal dendrites and the apical dendrites of pyramidal cells in hippocampal CA1 region are dependent on behavioral state. To further study the modulation of neuronal transmission by behavioral state, double pulses of electrical stimulation with interpulse interval (IPI) at 20, 30, 50 and 100 msec were delivered to Shaffer collaterals in the chronically implanted rat preparation during each of four behavioral states: immobile awaking (IMM), voluntary walking (WLK), slow-wave sleep (SWS) and REM sleep (REM). The paired pulse index (PPI, ratio of the amplitude of second population spike to that of first one) was classified according to the amplitude of first population spike (P1). The results showed that in a certain range of P1 amplitude the population spike of second pulse was significantly greater than first one (PPI>1) during WLK and REM at an IPI of 30, 50 and sometimes 100 msec. On the contrary, PPI<1 was observed during IMM and SWS for the same IPIs. If the IPI was decreased to 20 ms, the second population spike would be smaller than first one in most cases no matter which of the four behavioral states the rat was in. These results indicate that facilitation is the dominant response during WLK and REM, but inhibition is the dominant response during IMM and SWS. Besides, The PPI increased significantly as IPI increased from 20 to 50 msec for a given averaged P1 value for each of four behavioral states, which suggests that the ratio of facilitation to inhibition became greater as IPI prolonged. (Supported by NS25383 and NSERC).

514.10

SHORT-TERM CHANGES IN EEG-DESYNCHRONIZED STATES AFTER IBOTENIC INJECTION INTO INTRALAMINAR THALAMIC NUCLEI. G.Marini*, I.Gritti & M.Mancia (SPON: European Neuroscience Association). Istituto Fisiologia Umana II and *C.N.R.-ITBA, via Mangiagalli 32-Milano (Italy)

In this study we have examined the short-term effects (2 days) on the behavioral states of bilateral injections of ibotenic acid into the intralaminar thalamic complex.Nine cats,implanted with sleep recording electrodes, were used. Under ketamine anesthesia the animals received the excitoxin (50µg/µl-1.8µl) stereotaxically in the rostral (centralis lateralis,CL) or caudal (centrum medianum,CM) nuclei.When recovering from anesthesia, both groups of animals overacted to sensory stimuli and displayed a highly aroused behavior, associated with saccades and fast desynchronized EEG patterns.This acute enhancement was followed by a decrease in the amount of both EEG-desynchronized states (wake and REM sleep) and REM saccades,even though the amount of REM sleep increased in CL-, while decreased in CM-lesioned cats. The results support evidence of the role of intralaminar complex in tonic processes of cortical activation as well as in gaze mechanisms, suggesting, however, different functions of CL and CM nuclei in REM sleep.

514.11

SLEEP/WAKE ACTIVITY OF VENTROBASAL COMPLEX NEURONS IN THE RAT. G.A. Marks and H.P. Roffwarg. Dept. Psychiatry U.T. Southwestern Med. Sch., Dallas, Tx 75235.

The paucity or absence of GABAergic interneurons (G-I cells) in the ventrobasal complex (VB) of rat constitutes a specialization in thalamic cytoarchitecture that can be useful for the study of the functional roles of different neuronal populations in thalamus. We now report on the preliminary analysis of spontaneous activity of extracellularly recorded neurons in the VB of freely moving, unanesthetized Long Evans Hooded rats and compare it to activity obtained in the lateral geniculate nucleus (LGN), a relay nucleus with G-I cells, and thalamic reticular nucleus (TRN), a nucleus providing GABAergic inhibition to relay nuclei. VB cells tend to discharge more slowly than LGN relay cells but express a similar proportional change across states with the highest mean rates of discharge occurring during waking and REM sleep and lowest during SW sleep. Both cell populations increase rate in SW sleep at the transition to REM sleep. State-related discharge patterns differ. LGN cells fire in a regular burst-pause pattern in SW sleep and tonically in REM and waking. VB cells express more single spike activity in SW and are more bursty in REM. Comparing the activity of VB cells to somatosensory receptive cells of the TRN, we find little to relate them reciprocally. As a group, TRN cells do not change mean discharge rate with state except for increased rate in SW sleep at the transition to REM sleep. All TRN cells express marked pattern changes with strong rhythmicity across states. VB cells discharge with poor rhythmicity and not in the frequencies exhibited by TRN cells.

We conclude that the absence or presence of G-I cells can have an effect upon the sleep/wake related activity of thalamic relay cells. Interneurons may play a role in the control of discharge pattern and rhythmicity. No evidence is found to support a role of TRN in mediating sleep/wake activity in thalamic relay nuclei.

514.12

FIRING OF POSSIBLY CHOLINERGIC NEURONS IN THE LATERODORSAL TEGMENTAL NUCLEUS OF THE RAT DURING WAKEFULNESS AND SLEEP Y. Kayama and M. Ohta* Dept. of Physiology, Fukushima Medical College, Fukushima 960-12, Japan

To examine a hypothesis that cholinergic neurons in the laterodorsal (LDT) and pedunculopontine tegmental nuclei play a key role in shift or maintenance of sleep-waking state, single neuronal activity was recorded in the LDT of rats whose head was fixed painlessly with a stereotaxic device for chronic preparation, sleep-deprived beforehand.

Our previous study in anesthetized rats (Kayama & Ogawa Neurosci.Lett. 77:277-282,1987) showed that, among neurons encountered in the LDT, those firing broad spikes must be cholinergic. We could record from the same broad-spike neurons in this study. Some of these neurons were very silent during wakefulness, increased their firing slightly when the animal fell asleep, and during paradoxical sleep they had slowly fluctuating, high-rate discharge (up to 30 spikes/sec on a phase of the highest rate). Others had regular tonic firing of ≤5 Hz during wakefulness, which decreased during slow wave sleep and almost stopped during paradoxical sleep. The changes in firing usually preceded (2-20 sec) the changes in EEG (syn- or desynchronization).

These results suggest that 1) the LDT is a collection of cholinergic neurons heterogenous in nature, and 2) the cholinergic neurons may play an important role in induction and maintenance of high activity level of the upper brain during wakefulness and paradoxical sleep.

514.13

DESIPRAMINE- INDUCED REM SLEEP SUPPRESSION: EVIDENCE FOR A CENTRAL ALPHA- 1 ADRENERGIC MECHANISM. R.J.Ross, P.J.Gresch*, W.A.Ball*, L.D.Sanford* and A.R.Morrison. Dept. V.A.M.C. and Depts. of Psychiatry and Animal Biology, Univ. of Penna. Schools of Med. and Vet. Med., Phila., PA 19104.

Acute norepinephrine (NE) uptake blockade by desipramine (DMI) suppresses REM sleep (REMS) in the cat and other species. To understand the underlying adrenergic receptor mechanism(s), the effect of co-administering the α-1 antagonist prazosin or the β antagonist propranolol was investigated. Four cats were implanted with EEG, EOG, EMG, and lateral geniculate electrodes. Six- hour polygraphic recordings at baseline (placebo capsule, 3cc i.p. saline), after 1 mg/kg p.o. DMI plus i.p. saline, and after DMI plus prazosin (0.01, 0.03, or 0.1 mg/kg i.p.) were compared. Study condition order was counterbalanced, and trials were separated by 1 week. REM percentage (REMS time/total sleep time) was reduced by DMI (26.1 vs. 3.1, $p<0.001$) and increased again with prazosin co-administration at successively higher dosages (3.5 vs. 10.9, $p<0.1$; 3.1 vs. 12.2, $p<0.05$; 3.1 vs. 14.0, $p<0.05$). Propranolol (5 mg/kg i.p.) had no significant antagonistic effect, nor did the peripherally- acting hypotensive agent hydralazine (0.5 mg/kg i.p.). Thus, a central α-1 mechanism partially explains DMI's REMS suppressant effect. The results are consistent with the finding in narcoleptic dogs that cataplexy, which may be comparable to REMS atonia, has α-1 inhibitory modulation (Mignot, E. et al., J. Clin. Invest., 82:885, 1988). Supported by D.V.A. Med. Res. Serv., MH42903 and MH18825.

514.14

EFFECTS OF p-CHLOROPHENYLALANINE ON THERMOREGULATION AND SLEEP IN RATS AT SEVERAL AMBIENT TEMPERATURES. H.Li* and E.Satinoff. Psychology Dept., Univ. of Illinois, Champaign, IL 61820

p-chlorophenylalanine (PCPA), a relatively specific serotonin depletor, has been reported to cause hyposomnia for several days when injected i.p. We studied the participation of serotonin in the relationship between thermoregulation and sleep/waking in a 12:12 light/dark photoperiod. Telemetered body temperature (Tb) and sleep were recorded in male rats for 7 days after i.p injection of saline (at lights-on of the second day) and PCPA (300 mg/kg, at lights-on of the third day) at three ambient temperatures (Ta): 20 (n=7), 30 (n=7), and 32°C (n=8). At Ta 20°C, Tb dropped 2.3°C in the first 6 hr post-PCPA, and the amplitude of the Tb rhythm was lower than normal for the next 3 days. At Ta 30°C, there was no initial drop in Tb and Tb amplitude was attenuated for 2 days. At Ta 32°C, there were only minor changes in Tb. The effects of PCPA on sleep were independent of both Ta and Tb. In the light, REM sleep was depressed immediately and remained lower than normal for 3 days. Slow-wave-sleep was depressed for 2 days beginning 24-hr post-PCPA. These results suggest that the mechanisms by which serotonin depletion affects Tb and sleep are different.

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514.15

HYPOTHALAMUS AND SLEEP: TIME-COURSE OF SEROTONINERGIC CHANGES. M.G. De Simoni*°, L. Imeri*, R. Giglio*, A. Vezzani° and M. Mancia*. Inst. of Human Physiology, Milan I-20133 and °Inst. "Mario Negri", Milan I-20157, Italia.

Changes in serotonin (5HT) system in the anterior (MPA) and posterior (LH) hypothalamus have been studied in relation to sleep (S) and wakefulness (W). In vivo voltammetry and polygraphic recordings were performed simultaneously in freely moving rats by means of a telemetry system applied to voltammetric recordings.

The extracellular levels of the 5HT metabolite 5-hydroxyindolacetic acid (5HIAA) significantly increase with W (maximal increase: 15 ± 3.6 % in MPA and 15 ± 4.2 % in LH) and decrease with S (slow wave and paradoxical S; maximal decrease: -16 ± 3.7 % in MPA and -20 ± 3.1 % in LH). In both areas these changes begin some minutes before S or W are fully established. The changes related to S come significantly earlier in MPA than in LH and this difference is present only in the dark period. Bilateral lesions of MPA (0.5 ug/100 nl of kainic acid) induce a persistent increase of W during dark periods underlying the involvment of MPA in sleep control in relation to the light-dark cycle.

514.16

SENSITIVITY AND SELECTIVITY OF IN VITRO SEROTONERGIC RESETTING OF THE MAMMALIAN SUPRACHIASMATIC CIRCADIAN CLOCK. R.A. Prosser, J.D. Miller, and H.C. Heller*. Dept. Biological Sciences, Stanford University, Stanford CA 94305.

The suprachiasmatic nuclei (SCN) contain a circadian oscillator that produces a 24 hr rhythm of spontaneous electrical activity when isolated *in vitro*. We are using the SCN slice preparation to investigate the role of the serotonergic projection to the SCN from the raphe nuclei.

Brain slices containing the SCN were prepared from adult male Wistar rats housed in 12:12 LD. At a designated circadian time (CT), the slice perifusion medium was replaced for 1 hr with medium supplemented with the non-specific serotonin agonist quipazine or antagonist metergoline. The averaged electrical activity of single SCN neurons monitored on day 2 *in vitro* was compared to that of untreated slices (mean time-of-peak = CT 6.0±0.32 hr, N=5; CT 0 = lights-on) to determine whether the treatment shifted the SCN clock.

We determined that 1) quipazine permanently resets the SCN clock; 2) this effect depends on the CT of treatment, inducing 3-4 hr advances at CTs 6 and 9, 2-4 hr delays at CTs 15, 18 and 21, and little or no effect at CTs 0,3 and 12; 3) the ED_{50} for quipazine at CT 6 is 0.5 uM; 4) metergoline also resets the clock but at different CTs from quipazine; and 5) metergoline blocks phase-shifts induced by quipazine at CT 6. Together these results suggest that the SCN clock is directly sensitive to phase resetting by serotonergic ligands, and that this sensitivity is both dose- and time-specific.

514.17

EFFECT OF YOHIMBINE ON THE SLEEP-WAKE PATTERN OF LEAN AND OBESE ZUCKER RATS. **S. DeMesquita, K.A. Burgess* and E. Schoene*. Department of Physiology, Marshall Univ Sch of Med, Huntington, WV 25755-9340**

The sympathetic nervous system, which regulates not only feeding behavior but sleep, metabolism and energy balance, appears to be decreased or inhibited in the obese Zucker rat (Holt and York, Brain Res. 481:106-112,1989). Zucker rats were implanted with EEG and EMG electrodes and monitored for sleep-wake pattern before, during and after the infusion of the α_2-adrenoreceptor antagonist, yohimbine (YOH). YOH was infused continuously for 5 days (10mg/kg/day) s.c. in lean (N=5, 578g) and obese (N=6, 743g) Zucker rats, using miniosmotic pumps. Total sleep time % increased significantly (26%) in the obese rat during YOH and remained elevated following YOH withdrawal. Rapid Eye Movement (REM) sleep % was unchanged during YOH in both groups, however YOH withdrawal caused a 30% and 39% increase in REM% in lean and obese rats respectively, due to a 60% increase in REM period frequency. YOH and its withdrawal were associated with a significant rise in both Non-REM and REM sleep time in the obese rat, this effect was evident to a lesser extent only during YOH withdrawal in the lean rat.

514.18

THE D-1 DOPAMINE RECEPTOR MODULATES REM SLEEP IN THE RAT. M. Trampus , N. Ferri* and E. Ongini. Research Laboratories, Schering-Plough S.p.A., I-20060 Comazzo, Milan, Italy

The D-1 agonist SKF 38393 and the selective D-1 antagonist SCH 23390 were studied for their effects on sleep-waking cycle in the rat by using EEG techniques. SKF 38393 (0.1-10 mg/kg s.c.) dose-dependently reduced rapid eye movement (REM) sleep and enhanced wakefulness (W). The drug affected REM significantly over a low dose range (ED50 = 0.4 mg/kg) at which W remained unchanged and the characteristic grooming was not apparent. Effect on REM was characterized by a decreased number of episodes and no changes of latency to the first episode. SCH 23390 (0.0003-0.3 mg/kg s.c.) enhanced the amount of REM by increasing both number and average duration of episodes. There was also a moderate increase of non-REM sleep which was less marked than that occurring for REM. Given at 0.003 mg/kg, SCH 23390 fully prevented reduction of REM induced by SKF 38393 (0.3-3 mg/kg).
Considering the low dose range at which the D-1 agonist and the D-1 antagonist were effective on REM, it is suggested that D-1 receptors might have an important role in the regulation of REM sleep process.

514.19

RAPID GONADAL RECRUDESCENCE AND BODY AND LIPID MASS INCREASES WITH HYPOTHALAMIC LESIONS IN PHOTOREGRESSED SIBERIAN HAMSTERS. M. P. Maharaj*, T. G. Youngstrom and T. J. Bartness. Depts. of Psychology and Biology, Georgia State University, Atlanta, GA 30303.

We previously found that combined pinealectomized (PINX) and SCN lesioned (SCNx), photoregressed hamsters had recrudesced testes within 5 wks, but not intact or PINX-only controls. The purpose of the present experiment was to determine if this result was simply due to damage of retinal-pineal circuitry components. Photoregressed hamsters were PINX, SCNx, given paraventricular nucleus lesions (PVNx) or left intact. Blood was sampled weekly for 5 wks at which time testes and epididymal fat (EFAT) were harvested, circadian rhythms of wheel-running assessed and lesions histologically confirmed. Hamsters with PVNx hits and SCNx misses (mostly caudal and dorsal to the SCN) had increased testes, EFAT and body weights, increased food intake, normal activity patterns, and progressively increasing and marked serum PRL, but not FSH levels. In contrast, PINX, SCNx hits, PVNx misses and intact controls had typical short day values and SCNx hits had arrhythmic activity patterns. These results suggest an area caudal and dorsal to the SCN, and extending to and including the PVN, is involved with maintaining short day responses under these conditions and may also inhibit PRL release in long photoperiods based on preliminary lesion data.

514.20

COMPARISON OF EFFECTS ON SLEEP OF ADENOSINE AND ADENOSINE ANALOGS MICROINJECTED TO THE STRIATUM AND PREOPTIC AREA OF RATS. S.R. Ticho, M. Lekovic*, C. Vugrincic*, E. Dziennik*, and M. Radulovacki. Dept. of Pharmacology, University of Illinois, Chicago, IL 60612.

Adenosine A2 receptors are located in the olfactory tubercle, nucleus accumbens and striatum while adenosine A1 receptors show a heterogeneous distribution throughout the CNS. To localize adenosine's behavioral effects within different brain areas, we compared the effects on sleep of adenosine and adenosine analogs microinjected to the striatum and preoptic area of the rat. Polygraphic recordings were examined during a six hour period of the light cycle. Microinjections to the preoptic area were 0.5ul while injections to the striatum were 1.0μl. All three of the drug treatments, ADO (0.025M, n=10), a selective ADO A1 receptor agonist (CPA, 0.001M, n=5), and a nonselective ADO A1/A2 receptor agonist (NECA, 0.002M, n=7) microinjected to the preoptic area increased deep slow-wave sleep (SWS2) (27%, 27%, 48%) ($p<0.05$) respectively as compared to saline controls. Furthermore, ADO and NECA caused a significant increase in total sleep (13%, 16%) ($p<0.05$). In contrast, no changes in sleep parameters were observed when these drugs (ADO, n=6) (CPA, n=6) (NECA, n=6) were microinjected to the striatum. We observed a 20% increase SWS2 with NECA, this increase did not attain significance.

514.21

SOMNOGENIC AND PYROGENIC EFFECTS OF THE IMMUNOSTIMULANT FK-156. **K.A. Serpa and L.T. Meltzer, Dept. of Pharmacology, Parke-Davis Pharmaceutical Research Division, Warner-Lambert Co., Ann Arbor, MI 48105.**

Muramyl peptides (MP) enhance sleep in experimental animals. Muramyl dipeptide (MDP; N-acyl-muramyl-L-alanyl-D-isoglutamine), originally synthesized as an immunostimulant, is also somnogenic and pyrogenic. Additionally, a MP is the active component of Urinary Sleep Factor S. FK-156 (D-lactyl-L-alanyl-D-glutamyl-(L)-meso-diamino-pimelyl-glycine) and FR-40929 (L-alanyl-D-glutamyl-(L)-meso-diamino-pimelic acid) are MP analogs which lack a sugar moiety. FK-156 is a potent, while FR-40929 is a weak, immunostimulant. In order to better understand the structural requirements for different pharmacological activities we compared the somnogenic and pyrogenic effects of FK-156, FR 40929 and MDP in rabbits. MDP increased slow-wave-sleep (SWS) and body temperature (BT) at doses of 25-100 µg/kg s.c. FK-156, 100 µg/kg s.c., induced changes in SWS and BT that were comparable to 100 µg/kg of MDP. In contrast, FR-40929 was less potent than MDP and FK-156, with a threshold dose for increasing SWS and BT of 0.3 mg/kg s.c. These data indicate that a sugar moiety is not necessary for the somnogenic and pyrogenic effects of MP analogs.

NEUROTOXICITY: MPTP

515.1

METHAMPHETAMINE AND MPTP PRODUCE CHANGES IN PCP AND NMDA RECEPTOR BINDING IN MOUSE BRAIN. S.F. Ali, S.H. Lee*, J.F. Bowyer and W. Slikker, Jr.*. Div. of Reprod. & Develop. Tox., National Center for Toxicological Research, Jefferson, AR 72079.

In rodents and primates methamphetamine (METH) and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) are known to be toxic to presynaptic dopamine (DA) terminals. This study was designed to evaluate the in vivo effects of toxic doses of METH and MPTP on monoamine levels and n-methyl-D-aspartate (NMDA) and phencyclidine (PCP) receptors in mouse brain. Adult male C57/B6N mice were injected four times with 0 or 10 mg/kg METH, i.p. at 2 hr intervals or single injections of 0 or 25 mg MPTP/kg, i.p. Animals were sacrificed 3 days later. NMDA (^{3}H-CGS-19577) and PCP (^{3}H-TCP) receptor binding were analyzed in cerebral membrane preparations using filtration techniques and monoamine concentrations in striatum by HPLC/EC. Dopamine levels in striatum were significantly decreased (40-50%) after METH or MPTP treatment. In vivo METH and MPTP significantly decreased PCP but not NMDA receptor binding. In vitro MPTP (1-1000uM) produced a concentration dependent decrease in PCP and NMDA receptor binding but METH produced a decrease in PCP binding only at high concentrations (500 and 1000 uM). These data demonstrate that neurotoxicity induced by METH or MPTP, as indicated by decreasing DA levels, results in reduced ligand binding to the PCP or NMDA receptor complex.

515.2

METHAMPHETAMINE- AND 1-METHYL-4-PHENYL-1,2,3,6-TETRAHYDROPYRIDINE-INDUCED DOPAMINERGIC NEUROTOXICITY: MODULATION BY VARIOUS PRETREATMENTS. M.S. Saporito, C. Konradi, L. Manzino, P.K. Sonsalla and R.E. Heikkila. UMDNJ-Robert Wood Johnson Medical School, Piscataway, N.J. 08854.

Methamphetamine is a potent dopaminergic neurotoxin in the nigrostriatal tract of mice. This neurotoxicity is dependent on dopamine synthesis and release and is blocked by dopamine receptor antagonists such as haloperidol and by the non-competitive NMDA receptor antagonist MK-801. It has previously been reported that methamphetamine pretreatment leads to partial protection against a subsequent challenge dose of methamphetamine. In the present study, we have evaluated the effect of various pretreatment regimens on methamphetamine- and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced dopaminergic neurotoxicity. Mice were pretreated with methamphetamine or cocaine for four days and challenged on the fifth day with either methamphetamine or MPTP. Dopaminergic neurotoxicity was evaluated several days later by quantifying neostriatal dopamine content and tyrosine hydroxylase activity. A low dose pretreatment regimen with methamphetamine led to a nearly complete protection against subsequent methamphetamine-induced neurotoxicity. In contrast, cocaine pretreatment potentiated both methamphetamine- and MPTP-induced neurotoxicity. Potential mechanisms for these observations will be discussed.

515.3

DOPAMINE DEPLETION BY INTRASTRIATAL INJECTION OF MPTP N-OXIDE. Y.S. Lau, Y.K. Fung, K.L. Trobough*, J.R. Cashman* and J.A. Wilson. Creighton Univ., Omaha, NE 68178, Univ. of Nebraska, Lincoln, NE 68583 and Univ. of Calif., San Francisco, CA 94143.

MPTP N-oxide is the major peripheral metabolite found in vivo following systemic injection of the dopaminergic neurotoxin, MPTP (Lau et al., Life Sci. 43:1459, 1988; Chiba et al., JPET 246:1108, 1988). Despite its presence and high concentration, the properties of this potentially neurotoxic metabolite have not been described. MPTP, MPTP N-oxide (15 μg/side) or saline was injected bilaterally to the caudate-putamen of male C57/BL mice. Five hours later, the striatal dopamine (DA) levels in mice treated with MPTP (8.6$\pm$1.5 ng/mg) and MPTP N-oxide (11.0$\pm$1.1 ng/mg) were significantly lower than in the control mice (15.9$\pm$0.6 ng/mg). In vitro incubation of striatal homogenates with MPTP or MPTP N-oxide (0.89 mM) for 5 hours revealed that there was a time-dependent conversion of MPTP to MPP^+, which could be blocked by pargyline (0.01 mM). MPTP N-oxide remained unchanged in a 5 hour incubation. The central DA depleting action of MPTP N-oxide was correlated with its ability to cause [^{3}H]DA release in striatal synaptosomes. MPTP N-oxide did not alter the electrophysiologically recorded field potential in nigro-striatal slices. Our data show that MPTP N-oxide can directly cause the chemical depletion of striatal DA without modifying the characteristics of synaptic transmission. Supported by the Health Future Foundation.

515.4

NEUROTOXICITY OF MPTP-3-OL, AN MPTP ANALOG, TOWARD MOUSE STRIATAL DOPAMINE NEURONS. S. K. Hemrick-Luecke*, D. W. Robertson, J. H. Krushinski, Jr.* and R. W. Fuller. Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN 46285.

In CFW mice, 1,2,3,6-tetrahydro-1-methyl-4-phenyl-3-pyridinol (MPTP-3-OL) caused a dose-dependent depletion of striatal dopamine and its metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) 1 week after the last of 4 daily s.c. doses. MPTP-3-OL injected at 80 mg/kg s.c. resulted in a 71% depletion of dopamine, a 70% depletion of DOPAC and a 51% depletion of HVA in striatum and 56% depletion of norepinephrine in the frontal cortex of mice. MPTP-3-OL, like 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), is converted to 1-methyl-4-phenylpyridinium (MPP+). One hour after 80 mg/kg MPTP-3-OL injected s.c., MPP+ concentrations in the brain of mice were 7.41 µg/gm. Deprenyl pretreatment antagonized the depletion of brain catecholamines after MPTP-3-OL injection and inhibited the formation of MPP+ in whole brain of mice. These data are similar to those observed after MPTP injection into mice. In vitro, MPTP-3-OL was a substrate for monoamine oxidase type B from mouse brain and liver, being oxidized at a slower rate than MPTP. Deprenyl (10 µM) inhibited both MPTP and MPTP-3-OL oxidation in vitro. These data indicate MPTP-3-OL is a neurotoxic analog of MPTP with a unique structure, having a hydroxy substituent on the 3-position of the tetrahydropyridine ring of MPTP.

515.5

DOSE-DEPENDENT EFFECTS OF 2'-CH_3-MPTP ON MONOAMINERGIC SYSTEMS IN MICE: TYROSINE HYDROXYLASE AND GFAP IMMUNOCYTOCHEMICAL STUDIES. L. Gordon, X.L. Chen and M. Gupta. Dept. of Anatomical Sci. & Neurobiology, Univ. Louisville Sch. Med., Louisville, KY 40292.

Previous studies from this laboratory have shown that 2'-CH_3-MPTP produces a significant decrease in the number of tyrosine hydroxylase (TH)-positive neurons in substantia nigra and the ventral tegmental area. The present studies were undertaken to investigate if additional monoaminergic nuclei including locus coeruleus and the dorsal raphe dopaminergic neurons are also affected and if these effects are dose-dependent. Young adult male C57BL/6 mice were given multiple injections of 2'-CH_3-MPTP (7.5 and 10mg/kg i.p.) over a two day period. Three days later, control and treated mice were anesthetized and perfused with the fixative. Adjacent 40µm thick serial sections through the brain were stained immunocytochemically for TH and GFAP. The number of TH-positive neurons were quantitated in the SN, VTA, A8, locus coeruleus and dorsal raphe as well as GFAP-immunoreactive astrocytes in the striatum. The results show that 2'-CH_3-MPTP produced a statistically significant and a dose-dependent decrease in the number of TH-immunoreactive neurons in the SN, VTA and A8 followed by extensive gliosis in the striatum compared to the controls. These data demonstrate that 2'-CH_3-MPTP is much more toxic than MPTP HCl and affects several monoaminergic nuclei in the brain. Quantitative analysis on gliosis in the striatum is currently in progress. Supported by USPHS grant R29 NS24291 to MG.

515.6

LONG-TERM SURVIVAL OF 2'-CH_3-MPTP-TREATED MICE: TYRINE HYDROXYLASE AND GFAP IMMUNOCYTOCHEMICAL STUDIES. M. Gupta and X.L. Chen. Dept. of Anatomical Sci. & Neurobiology, Univ. Louisville Sch. Med., Louisville, KY 40292.

Previous studies from this laboratory have shown that dopaminergic neurons in the substantia nigra, ventral tegmental area and the A8 cell group are reduced significantly after short-term survival following 2'-CH_3-MPTP treatment in mice. In the present studies, young adult male C57BL/6 mice were treated with 2'-CH_3-MPTP over a two day period (total dose 30mg/kg i.p.) and examined 2 weeks, 2 months and 6 month after treatment. At the appropriate survival times, control and 2'-CH-MPTP-treated mice were anesthetized and perfused intracardially with 4% paraformaldehyde and 1.5% sucrose in 0.1M PO_4 buffer. Serial 40µm thick sections were cut through the entire brain; adjacent section were stained immunocytochemically for tyrosine hydroxylase (TH) and Glial Fibrillary Acidic Protein (GFAP). The number of TH-positive neurons were quantitated in the SN, VTA and A8. The results show a significant reduction in the number of TH-immunoreactive neurons in the SN and VTA up to 6 months after treatment followed by extensive gliosis in the striatum. Quantitative analysis of the GFAP-stained astrocytes in the striatum is currently in progress. The following will be discussed: (a) regeneration and plasticity of the dopamine neurons in the SN, VTA and A8 after long-term survival follwing treatment with 2'-CH_3-MPTP and (b) astrocyte proliferation in the striatum with long-term survival. Supported by USPHS grant R29 NS24291 to MG.

515.7

MOBILIZATION OF MACROPHAGES IN DOPAMINERGIC AREAS OF THE BRAIN AFTER ADMINISTRATION OF MPTP. A. Hess, C. Desiderio* and W. G. McAuliffe*. Dept. Neuroscience and Cell Biology, UMDNJ, RW Johnson Med. Sch., Piscataway, NJ 08854.

Immunohistochemical staining with Mac-1 antisera reveals microglial cells. The cells are numerous and located in gray and white matter in all areas of the brain. Cell bodies are samll, processes are elongated and spiked and appear like microglial cells after silver carbonate staining. After MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine),(mice, 50mg/kg s.c., one per day for 2 days, 3 days after last injection), Mac-1 staining reveals very intensely stained cells accumulated in the nucleus accumbens and restricted in the substantia nigra to the pars compacta. These are locations of dopaminergic cells, fibers and terminals, and this can serve as an illustration of the specificity of action of MPTP as a dopaminergic neurotoxin. These cells appear to be macrophages and have intensely-stained cell bodies and numerous short processes radiating out from the cell body. Since both microglia and macrophages have the same antigen type, we suggest that resident microglia inherent in the brain become reactive microglia after degenerative effects of MPTP on dopaminergic neuronal elements, and hypertrophy, become round, shorten their processes and become macrophages. The mobilization of macrophages after MPTP adds an additional factor the etiology of the pathophysiology of MPTP-induced neurotoxicity.

515.8

VITAMIN E DEPLETION DOES NOT POTENTIATE MPTP TOXICITY IN C57 BLACK MICE. John S. Althaus, Michael A. Burian*, Chris J. Hudson* and Philip F. VonVoigtlander, CNS Diseases Research, The Upjohn Co., Kalamazoo, MI 49001.

The current study was designed to test the importance of endogenous antioxidants in MPTP toxicity. The approach taken was to deplete vitamin E in C57 Black mice using a vitamin E deficient diet. MPTP was then administered at 10 or 30 mg/kg i.p. once daily for 5 consecutive days. The results showed that dietary vitamin E manipulation did not affect MPTP toxicity. In mice fed basal or vitamin E deficient diets for two weeks or twelve weeks, catechol levels of the striatum and tegmentum were depleted to the same extent by MPTP. The results also show that MPTP did not affect vitamin E concentrations in the striatum or tegmentum. The depletion of vitamin E by dietary restriction was not enhanced by MPTP administration.

Because vitamin E is an antioxidant which functions primarily in membranes, we proposed that MPTP toxicity does not appear to directly involve lipid peroxidation in this model. That is not to say that MPTP toxicity does not involve the generation of free radicals or oxidative stress. It does suggest that if these processes are involved in MPTP toxicity, then peroxidative damage to membrane lipids is not a major mediator of the damage caused to dopamine neurons.

515.9

THE METABOLIC EFFECTS OF MPP+ IN CULTURED CEREBELLAR GRANULE CELLS A.M. Marini, T.S. Nowak Jr, J.P. Schwartz, and I.J. Kopin.
Clinical Neuroscience Branch, NINDS, NIH, Bethesda, Maryland 20892.

We have determined, in a homogeneous population of cerebellar granule cells in culture, intracellular metabolite levels of adenosine triphosphate (ATP), phosphocreatine (PCr) and creatine, as well as medium glucose and lactate levels, following exposure to 50 micromolar MPP+. MPP+ depleted PCr to 20% of control levels within 30 minutes and it remained at this level for up to one week. The decrease in PCr was accompanied by an equimolar *increase in creatine.* *No significant* change in ATP levels occurred within the first 48 hours, but 50% decreases were observed after this time. There was also a marked decrease in medium glucose, with enhanced lactate accumulation. Neurons remained viable for at least 9 days in culture. Although glucose was repleted every three days after day 7 in culture, neuronal death occured after day 12 in culture. These results suggest that the neurons are sustaining their ATP levels through anaerobic metabolism of glucose in the presence of MPP+.

515.10

MITOCHONDRIAL RESPONSE IN N2AB-1 NEUROBLASTOMA CELLS AFTER EXPOSURE TO MPP+. S.J. Simmons [1], J.T. Hansen [2] and M. F.D. Notter [2] [1]Environmental Health Science Center and [2]Department of Neurobiology and Anatomy, University of Rochester, Rochester, NY 14642.

The effect of MPP+ on the N2AB-1 mouse neuroblastoma cell line was examined by electron microscopy with morphometric analysis and by the metabolic parameters glucose consumption and lactate production. Previously, we demonstrated that differentiated N2AB-1 cells are less sensitive to MPP+ toxicity than mitotic N2AB-1 cells as assessed by morphology, cell number and protein incorporation.

Mitochondrial area, ratio of mitochondrial area to cytoplasmic area(Ma/Ca), and percent damaged mitochondria were measured morphometrically. The control mitotic and differentiated N2AB-1 cells were equivalent for all three parameters. After MPP+(100uM) exposure for 24hr, the Ma/Ca increased from 9% to 15%, the mitochondrial area tripled, and 85% of the mitochondria were damaged in both mitotic and differentiated cells. Mitochondria of both mitotic and differentiated cells *were equally effected by MPP+ exposure.*

Lactate production and glucose consumption were 300% of control after 24hr MPP+ exposure in both mitotic and differentiated cells. After 48hr exposure, total lactate production was 270% of control, while total glucose consumption was 240% of control in both phenotypes. A similar response was seen after exposure to the mitochondrial toxin Rotenone(2uM), indicating a loss of mitochondrial function and increased dependence on glycolysis for energy.

These data indicate that while mitotic N2AB-1 cells are more sensitive to MPP+ in whole cell response(cell death and decreased protein synthesis), the mitochondria of both mitotic and differentiated cells respond equally to the toxic insult, suggesting that total metabolic requirements of the cell play a role in the sensitivity to MPP+.

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515.11

4'-ALKYLATED ANALOGS OF 1-METHYL-4-PHENYLPYRIDINIUM ION ARE POTENT INHIBITORS OF MITOCHONDRIAL RESPIRATION. R.E. Heikkila, S.K. Youngster, M.R. Gluck*, and W.J. Nicklas
Dept. of Neurology, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ 08854

1-Methyl-4-phenylpyridinium ion (MPP^+), a major brain metabolite of the dopaminergic neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, is an inhibitor of Complex I of the mitochondrial respiratory chain. We have synthesized several analogs of MPP^+ containing various alkyl groups in the 4' position of the phenyl ring and have determined their capacity to inhibit the oxidation of NADH-linked substrates by intact mouse liver mitochondria. These compounds are considerably more potent inhibitors of respiration than MPP^+ itself, with the potency increasing as the length of the alkyl chain increases. The most potent inhibitor, the 1-methyl-4-(4'heptylphenyl)pyridinium species (IC_{50}=0.5 μM), is about 200 times as effective as MPP^+ itself (IC_{50}=110 μM). In addition, tetraphenylboron (TPB), a lipophilic anion, is able to increase the potency of all these MPP^+ derivatives. However, the relative degree of potentiation appears to decrease as the length of the 4'-alkyl chain increases. Thus, all of the MPP^+ analogs are closer in inhibitory potency in the presence of TPB then in its absence. These observations suggest that the presence of an aliphatic 4'-alkyl group increases the accessibility of the compound to its inhibitory site. These analogs should prove to be useful tools for studying the nature of the process whereby MPP^+ and its pyridinium analogs interact with Complex I to inhibit mitochondrial respiration.

515.12

STUDIES ON THE MECHANISM OF MPP^+-INDUCED NEUROTOXICITY USING *IN VIVO* DIALYSIS
A. Giovanni, M.S. Saporito, S.K. Youngster and R.E. Heikkila. Dept. Neurology, UMDNJ-Robt. Wood Johnson Med. Sch., Piscataway, NJ 08854.

The dopaminergic neurotoxicity of MPTP and several of its analogs depends on 1) bioactivation of the tetrahydropyridine to a pyridinium species via MAO and 2) active accumulation of the pyridinium into dopaminergic neurons via the dopamine uptake system. Additionally, evidence exists which suggests that the inhibition of mitochondrial oxidation of NADH-linked substrates by these pyridinium species is an important feature of the neurotoxicity. In this study, several MPP^+ analogs were tested for their capacity to act as substrates for the dopamine carrier and for their ability to inhibit mitochondrial respiration. Compounds were subsequently chosen for *in vivo* neurotoxicity studies which demonstrated ranges in potency to serve as a substrate for the dopamine carrier and to inhibit mitochondrial respiration. The neurotoxicity of MPP^+ and its analogs was determined after the central administration of the compounds via an *in vivo* dialysis probe followed by the measurement of stimulated extracellular dopamine levels. Neurotoxic MPP^+ analogs, like MPP^+ itself, caused an irreversible depletion of DA as demonstrated by the lack of a stimulated increase in extracellular DA brought about by an infusion of MPP^+ 24 hours after an initial exposure to the analog. Data from the *in vivo* dialysis studies strongly support the theory that the neurotoxicity of these compounds depends not only upon their ability to act as substrates for the DA carrier but also upon their capacity to inhibit mitochondrial respiration.

515.13

MECHANISM OF ACCUMULATION OF 1-METHYL-4-PHENYLPYRIDINIUM ION (MPP^+) INTO MOUSE BRAIN SYNAPTOSOMES. D.A. Di Monte, K.P. Scotcher*, I. Irwin*, L.E. DeLanney, and J.W. Langston. California Parkinson's Foundation and California Institute for Medical Research, San Jose, CA 95128.

Incubation of mouse brain synaptosomes (essentially devoid of contamination by extrasynaptosomal mitochondria) in the presence of MPP^+ resulted in its time- and concentration-dependent accumulation. Intrasynaptosomal concentrations of 79 μM and 106 μM were reached 10 and 30 min, respectively, after addition of 50 μM MPP^+. The uptake of MPP^+ into synaptosomes was relatively unaffected by the catecholamine uptake blocker mazindol; in contrast, the rate of MPP^+ uptake was greatly increased by tetraphenylborate, a lipophilic anion that facilitates the transport of permeant cations across membranes via a Nernstian concentration gradient. Furthermore, MPP^+ accumulation was significantly increased (by substituting NaSCN and KSCN for NaCl and KCl in the incubation medium) or decreased (by ouabain, veratridine or KCl) as a consequence of enhancing or lowering, respectively, the plasma membrane potential of synaptosomes. Data indicate that (1) MPP^+ present at concentrations in the 10^{-5} M range may cross neuronal membranes despite its charged chemical structure and without the need of a specific uptake mechanism, and (2) polarization of neuronal membranes may facilitate the accumulation of this toxic cation into nerve terminals.

515.14

MPP^+-TYPE NEUROTOXICITY OF A PYRIDINIUM METABOLITE DERIVED FROM HALOPERIDOL. H. Rollema, B. Subramanyam*, N. Castagnoli Jr*. Dept. Medicinal Chemistry, University Groningen, 9713 AW The Netherlands and Dept. Chemistry, Virginia Polytechnic Institute, Blacksburg, VA 24061.

Neurotoxic properties of a recently described (Subramanyam, B. et. al., *BBRC*, 166:238, 1990) pyridinium metabolite ($HALP^+$) of the neuroleptic agent haloperidol, were compared with those of the dopaminergic neurotoxin MPP^+. A rat intrastriatal microdialysis assay was employed to assess the potential toxicity of $HALP^+$. The test compounds (2mM MPP^+ and 2mM $HALP^+$) were perfused intrastriatally via the dialysis membrane for various time periods and the output of the neurotransmitters and metabolites under investigation was monitored continuously. A challenge perfusion with MPP^+ 24 hours later was used to determine the extent of irreversible nerve terminal damage caused by the perfusion with the test compounds the previous day. $HALP^+$ lacks the acute potent dopamine (DA) releasing effect of MPP^+. However, a 7.5 hour lasting $HALP^+$ perfusion compromises dopaminergic nerve terminals as shown by a small DA release induced by the challenge MPP^+ perfusion. The effects of $HALP^+$ on lactate production were also measured by microdialysis. A 1 hour perfusion with HALP+ caused an increase in lactate levels to about 150% of basal values, indicating that $HALP^+$, like MPP^+, inhibits mitochondrial respiration in vivo. These results show that various other types of quaternary pyridinium compounds, not closely structurally related to MPP^+, can cause dopaminergic toxicity. They also suggest a potential role of $HALP^+$ as a cause of persistent tardive dyskinesia after chronic use of haloperidol.

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515.15

TRANSGENIC MICE EXPRESSING THE HUMAN SOD GENE ARE RESISTANT TO MPTP-INDUCED TOXICITY.[1]S. Przedborski, [1]V. Kostic, [1]V. Jackson-Lewis,[2]E. Carlson*,[2]C.J. Epstein* [1]J.L. Cadet. [1]Columbia University, NY 10032 and[2]University of California, CA 94143.

MPTP causes significant depletion of striatal dopamine (DA) which is similar to that seen in Parkinson's disease (PD). Free radical (FR) toxicity has been suggested to be involved in the pathogenesis of both PD and MPTP toxicity. Superoxide dismutase (SOD) is a key enzyme of the antioxidant system that protects cells from the hazards of FR. In order to further evaluate the role of FR in MPTP-induced toxicity, we tested the possibility that transgenic (Tg) mice which express the human SOD gene (Epstein et al., PNAS 84:8044, 1987) might be protected against the toxic effect of MPTP. Young white adult SOD-Tg mice and their non-Tg littermate were injected with MPTP (30 mg/kg, i.p. for 3 days) while SOD-Tg and non-Tg control mice received saline injections. Five days after the last injections, the mice were sacrificed and striatal DA, serotonin (5-HT) and their metabolites (DOPAC, HVA and 5-HIAA) were determined. MPTP reduced DA level (-56%; $P<0.01$) in the non-Tg mice. DOPAC and HVA levels were also significantly decreased. In contrast, MPTP did not affect DA, DOPAC, or HVA levels in the SOD-Tg mice. In both MPTP-treated groups, 5-HT and 5-HIAA levels were not different from controls. These results indicate that increased SOD activity may prevent the toxicity of MPTP in mice presumably by scavenging FR formed after MPTP administration. NHCHHD HD-17001, PDF.

515.16

ACUTE MPTP INDUCES C-FOS GENE EXPRESSION IN MOUSE BRAIN. A.M. Duchemin, K.P.Gudehithlu, N.H. Neff and M. Hadjiconstantinou. Depts of Pharmacology and of Psychiatry, The Ohio State University College of Medicine, Columbus, OH 43210.

c-Fos protein acts as a transcriptional regulatory factor for a number of genes expressed in the brain. Its synthesis can be increased by various stimuli that modify neuronal function. In this report, we show that the neurotoxin, MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine), induces a transient expression of c-fos gene in the mouse brain.

MPTP was injected i.p. into mice and total RNA from several areas of the brain extracted at different time intervals following the injection. c-fos mRNA was assayed by Northern blot hybridization with a nick-translated XhoI-NcoI restriction fragment from the pc-fos (mouse)-3 gene obtained from ATCC. c-fos mRNA increases were detectable with doses of MPTP as low as 10 mg/kg and the changes increased with the dose of MPTP. The dose-related changes were similar for several brain regions. The time-course of the induction of c-fos mRNA was studied after a dose of 50 mg/kg MPTP. In the striatum, c-fos mRNA increased 20 min after injection, was maximal at 40 min and decreased to control values at 90 min. In the hippocampus, the increase of c-fos mRNA was delayed: the content was maximal at 50 min, remained elevated at 90 min and decreased to control levels at 2 hrs. The D1 and D2 dopaminergic receptor antagonists, haloperidol (10 mg/kg), sulpiride (150 mg/kg), and SCH 23390 (10 mg/kg), injected i.p. 30 min before MPTP, did not prevent the increase of c-fos mRNA. These results suggest that the response to MPTP is apparently unrelated to interaction with dopamine receptors.

515.17

EARLY-LIFE MPTP EXPOSURE SLOWS AGE-RELATED LOSS OF SUBSTANTIA NIGRA NEURONS. W.G. Tatton, C.E. Greenwood, N.A. Seniuk, P. Salo, D. Holland* and M.Kwan*. Departments of Physiology and Nutritional Sciences, University of Toronto, Toronto, Ontario, Canada. M5S 1A8.

We showed that four populations of immunocytochemically-identified monoaminergic neurons display different rates of age-related loss across the lifespan in C57Bl mice (Tatton et al, Soc. Neurosci.15:160,1989). Neuronal loss was exponential (% Loss= $e^{-A*Age\ in\ Wks + B)}$ with 99% confidence limits from ± 1 to 3%. Calne & Langston postulated that toxin-induced and age-related neuronal loss sum to determine the time course of disabilities found in some neurodegenerative diseases (Lancet 2:1457,1983). We tested this hypothesis by treating C57Bl mice with 60 or 150 mg/kg of MPTP or saline at 8 wks of age and examining substantia nigra compacta (SNc) neurons at 52 or 88 wks of age using immunocytochemistry for tyrosine hydroxylase (TH), neuron specific enolase and neurofilament proteins together with the retrograde transport of fluorogold. Measurement of the % area of TH+ tissue in the ipsilateral striatum normalized against the number of TH+ SNc somata (counted from alternate sections through the whole nuclei) was used as a measure of relative terminal axonal length. Comparison of the numbers of surviving TH+ SNc neurons in MPTP treated mice to summed values for the age-related loss plus the MPTP dose-dependent loss of TH+ SNc neurons at 20 days after MPTP exposure (Seniuk et al, Br. Res. 1990, in press) showed that MPTP treatment at 8 wks of age markedly slows the rate of loss of neurons surviving MPTP exposure (A constant = 0.0130 for saline but = 0.0068 for SNc neurons surviving early life MPTP, $p> .001$). Saline-treated and MPTP-treated aged SNc neurons showed average increases of somal crossectional area, estimated terminal axonal length/SNc neuron and TH immunodensity/unit area of soma relative to those in 8 wk old mice that are proportional to the combined loss of neurons by toxic exposure and aging. The slowed age-related loss of SNc neurons surviving early-life MPTP exposure will be considered relative to somal hypertrophy, terminal axonal sprouting and alterations in the synthesis of specific proteins in the neurons . (MRC Canada grant MT5218)

CLINICAL CNS NEUROPHYSIOLOGY

516.1

A COMPARISON OF SPATIOTEMPORAL VOLTAGE DISTRIBUTIONS OF THE SECOND AND THIRD DIVISIONS OF THE TRIGEMINAL NERVE EVOKED POTENTIAL IN MAN. C. G. Widmer. Dental Research Center, Emory University School of Dentistry, Atlanta, GA 30322.

Mapping the initial cortical response to trigeminal nerve stimulation has been reported (Widmer, C. G., *J. Dent. Res.*, 66:117, 1987), but no study has examined spatiotemporal voltage distributions of different divisions within the same subject. Also, no study has mapped the muscle reflex potentials which may contaminate the evoked response. The purpose of this study was to map the evoked responses after stimulation of the greater palatine nerve (V_2) and mental nerve (V_3). Median nerve evoked potentials were used as a control.

Three subjects were stimulated with a 0.2 ms pulse at 1.2 Hz and averaged for 512 stimuli. Potentials from 28 scalp recording sites (modification of 10-20 International system) were amplified (bandpass 1-1000 Hz), acquired at 4000 Hz/channel, averaged by a computer acquisition system (BrainWave Systems) and stored on disk for subsequent mapping. Topographical activity maps were generated (BrainWave Systems) in the range of 14-30 ms for the median nerve and 4-20 ms for the trigeminal nerve and the initial cortical responses were identified and latencies were recorded.

Median nerve evoked responses occurred at 20.2 ± 1.4 ms with the greatest activity in the parietal region contralateral to the stimulation site. Both V_2 (12.1 ± 1.0) and V_3 (12.8 ± 1.1) distributions were highest in amplitude over the region of the somatosensory cortex (C5-T3, C6-T4) contralateral to the stimulation site. The V_2 distribution was located slightly superior to the V_3 initial cortical response consistent with known trigeminal topographical distribution in the somatosensory cortex. Muscle reflex potentials were identified *bilaterally* in the same region.

Supported by USPHS DE 06974.

516.2

FRACTAL DIMENSION OF AN EVOKED POTENTIAL. W.D. McCall, Jr. and C.G. Widmer. SUNY Buffalo, NY 14214 and Emory Univ., Atlanta, GA 30322.

If the evoked potential is deterministic and chaotic, it should have a fractal dimension. Our purpose was to obtain the fractal dimension of an evoked potential.

The median nerve in one subject was stimulated at 1.2 Hz with 0.2 ms pulses at 3 times sensory threshold for 256 trials while recording each response from 28 sites simultaneously. The correlation dimension (Grassberger and Procaccia, Physica D 9:189, 1983) was calculated for the 30 ms potential using recording sites as embedding dimensions. Straight lines were fit to the log-log display of the cumulative distribution of distances by a least-square-error algorithm. An acceptable fit had five or more points and a correlation coefficient of 0.980 or greater.

The correlation dimension increased until the 12th embedding dimension where the correlation dimension was 6.4. Since the stroboscopic Poincare section reduced the original dimension by one, we interpret our result as suggesting that the fractal dimension of the evoked potential was about 7.4.

Support by DE-07089 and DE-06974.

516.3

CHOLINERGIC EFFECTS ON HUMAN MIDLATENCY AUDITORY EVOKED RESPONSES. R.J.Strandburg, J.S.Buchwald, E.H.Rubenstein* and J.Schwafel*. Depts. of Psychiatry, Physiology & Anesthesiology, UCLA School of Medicine, Los Angeles, CA 90024

In contrast to the Pa component of the auditory middle latency evoked response (MLR), marked P1 abnormalities are observed in Alzheimer's disease, autism and schizophrenia. To further differentiate and characterize these two components, click elicited MLRs were recorded from 7 normal male volunteers receiving the cholinergic antagonist scopolamine followed by the agonist physostigmine. Pa (25-40 msec), Nb (40-50 msec) and P1 (50-65 msec) components obtained in these 2 conditions at a central scalp lead (CZ) were compared with immediately preceding control data recorded with click rates of 1, 5, 8 and 10/sec (P1 disappears at the faster rates while Pa is not affected).

Intravenous scopolamine eliminated the P1 and slightly increased Pa; subjects reported drowsiness but were awake with eyes open throughout the recordings. Subsequent injections of physostigmine resulted in a rapid reversal of the scopolamine effects: the subjects became alert, Pa decreased, and P1 returned to its control amplitude (and declined in response to rapid click rates). These data in conjunction with the results of related studies of MLRs in the cat suggest that the P1 generator system includes a cholinergic brainstem-thalamic component of the ascending reticular activating system.

(Supported by USPHS Grants HD05958 and HD04612)

516.4

DEPTH CORRELATES OF MIDLATENCY AUDITORY P1 IN THE CAT. J. Harrison, K. Tung* and J. Buchwald. Physiology Dept., Brain Res. Inst., and Mental Ret. Res. Ctr., UCLA, Los Angeles, CA 90024.

The "cat-P1", previously called by us "wave A", is a positive potential evoked by clicks, with a latency of 20-30 msec, recorded from the vertex. Like the human P1, it is absent with rapid click repetition rates (10/sec) and during slow-wave sleep but present during wakefulness and REM sleep. Both the P1 and cat-P1 are reversibly eliminated by scopolamine, a muscarinic cholinergic antagonist, and recover with physostigmine, a cholinesterase inhibitor. The human P1 is absent in a group of Alzheimer's disease patients, and the cat-P1 is abolished by bilateral lesions of the pedunculopontine tegmental nucleus (PPT) in the midbrain reticular formation. To test the hypothesis that the vertex-recorded cat-P1 is generated by PPT projections to the thalamus, we recorded from thalamic sites and tested midlatency click-evoked potentials with the effects of rapid repetition rates and cholinergic drugs. EEG was recorded from awake cats with clicks presented at a rate of 0.2/sec or 10/sec. Most of the depth-recorded potentials, many targeted for thalamic nucleus centrum medianum, were diminished or abolished by rapid click rates and scopolamine. Like the vertex cat-P1, the depth potentials sometimes recovered or were enhanced by physostigmine or carbachol. These data further support the hypothesis that the vertex cat-P1, and perhaps the similar human P1, reflect activity in a cholinergic system projecting from PPT to the thalamus. (Supported by USPHS HD05958 and NS25400.)

516.5

CORRELATION OF DICHOTIC LISTENING WITH CORTICAL AUDITORY EVOKED POTENTIALS IN PATIENTS WITH COMPLEX PARTIAL EPILEPSY. S.Khoshbin, B.J.Murawski*and C.S.Petrou*. Dept.of Neurology, Harvard Medical School, Boston, MA 02115.

Forty patients with history of partial complex seizures were studied using the dichotic listening test (Kimura, 1961), the Wechsler Adult Intelligence Scale, routine electroencephalography and cortical auditory evoked potentials. Correlation was found between these tests and information from neurological exams and radiographic studies. All patients with abnormal dichotic digits had abnormalities on cortical evoked potentials at N100, P200 or P300 components. The routine electroencephalogram was rarely informative. Abnormalities of dichotic digits correlated well with topographic mapping of evoked potentials with regard to dominant and non-dominant hemisphere findings. Good correlation was also found between discrepancies in verbal and performance scores on the Wechsler Adult Intelligence Scale and cortical auditory evoked potentials. Sample cases will be illustrated.

516.6

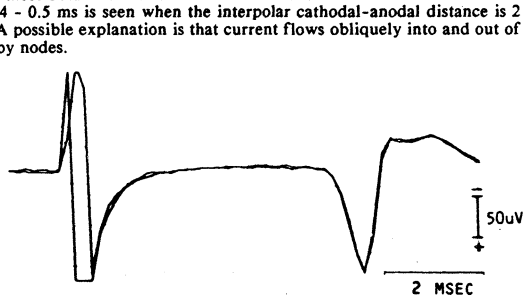
EFFECT OF DEXMEDETOMIDINE (D), ON VISUAL EVOKED POTENTIALS (VEPs) IN CHRONICALLY INSTRUMENTED CATS. K.A. Poterack* and W.T. Schmeling. Depts. of Anesthesiology and Pharmacology , Medical College of Wisconsin, Milwaukee, WI 53226

Inhalation anesthetics, including halothane (H), have been shown to modulate both the amplitude and latency of VEPs. D, an $alpha_2$-adrenergic agonist, produces profound sedative/anesthetic-like actions. The present study examined the effects of D (5, 10 µg/kg) and H (1-2%) on strobe flash VEPs of cats chronically instrumented with stainless steel electrodes to record from cortical (C) and lateral geniculate (LG) sites. A deep electrode was placed in the mesencephalic reticular formation (RF) for the production of cortical arousal. H produced an increased latency and decreased amplitude of the P1 peak recorded from C and LG. D administration, while producing clinical sedation and EEG slowing, resulted in preservation of the VEP waveform without significant change in amplitude or latency. RF stimulation after agent administration produced EEG changes consistent with arousal and return of VEP waveforms towards control. The receptor-specificity of the $alpha_2$ - agonists may produce sedation/unconsciousness without the global disruption of neural pathways produced by the inhalation agents. (Supported in part by PHS grants HL 36144, 1T 326MO8377 and VA Medical Research Funds).

516.7

EFFECTIVE ANODE AND CATHODE ARE VERY CLOSE TOGETHER WHEN STIMULATING PERIPHERAL NERVE WITH THE MAGNETIC COIL. P.J. Maccabee, V.E. Amassian, R.Q. Cracco, L. Eberle*, A. P. Rudell, K.S. Lai*. Departments of Neurology and Physiology, SUNY, Health Science Center, Brooklyn, N.Y. 11203.

We stimulated distal median nerve at the wrist using monophasic magnetic coil (MC) pulses and a novel hardware switching device which reverses current in the round and figure of 8 MC (Cadwell Laboratories). Thus, no errors are introduced by physically rotating the round MC through 180° to reverse current flow. Thenar motor unit responses were elicited at threshold intensities. No shift in latency was detected when the current in the figure 8 MC was reversed (Fig. 1) confirming our previous observations when the round MC was rotated. These data differ markedly from those obtained with conventional electrical stimulation where a shift of 0.4 - 0.5 ms is seen when the interpolar cathodal-anodal distance is 2 cm. A possible explanation is that current flows obliquely into and out of nearby nodes.



516.8

HUMAN NEUROMAGNETIC AND NEUROELECTRIC ALPHA FREQUENCY ACTIVITY REACTIVITY AND EVOKED FIELDS. C.C. Gallen, S. Hampson*, T.T. Yang*, F. Bloom, W. Young. Dept. of Neuropharmacology, Res. Inst. of Scripps Clinic, La Jolla, CA 92037.

A " moving window" approach was used to estimate the rates and timing of stimulus-related reductions in magnetoencephalographic (MEG) and electroencephalographic (EEG) intrinsic alpha frequency power in five paired (MEG probe located directly over EEG electrode) occipital sites. Serial recordings of 120 epochs of cycling central visual stimulus on/off in eight normal subjects allowed isolation of the two-second periods preceding and following onset of visual stimulus in each epoch. Waveforms were rectified and the average amplitude of alpha in an initial 100 msec window was calculated and plotted. Subsequently the window was "moved" in small increments and the average amplitude serially recalculated and plotted. This process produced a smoothed line reflecting fluctuations in alpha amplitude over time. Modelling studies with square wave and linearly sloped data revealed potential distortion of latency estimates for onset and cessation of reactivity. But, when certain specified characteristics were present, this measure showed utility as both a comparative measure of rate of change and for estimation of the midpoint of signal decline. As used in this study, the "moving window" method indicated maximal alpha reactivity in the first few hundred msec following stimulus onset, a period which overlapped multiple visual evoked waves. With limitations, the moving window approach allows estimation of the rate of decline of rhythmic activity.

516.9

BEHAVIORAL STATE SPECIFIC CHANGES IN THETA ACTIVITY: SCALP EEG VS. HIPPOCAMPAL DEPTH ELECTRODE RECORDINGS. J.L. Thompson, K.J. Meador*, D.W. Loring*, G.P. Lee*, D.W. King*, B.B. Gallagher, A.M. Murro*, J.R. Smith* and H.F. Flanigin*. Departments of Neurology and Surgery, Medical College of Georgia, Augusta GA 30912.

We previously demonstrated differential effects of behavioral state on human hippocampal EEG activity. In this study, we compared the percentage of theta activity recorded from a scalp electrode (Cz) to that recorded from a hippocampal depth electrode contralateral to the seizure focus in 13 patients with unilateral temporal lobe epilepsy (7 left focus, 6 right focus). Behavioral conditions incuded: resting eyes closed (RC), resting eyes open (RO), eyes open with auditory word activation (AW), eyes open with visuospatial activation (AV). Theta activity decreased significantly at both sites during RO and AV compared to RC, and during AV compared to AW ($p<0.02$). In the RC state, theta activity predominated in the scalp record, while delta activity predominated in the depth record. Theta activity decreased during RO ($p<0.05$) compared to AW in the depth record alone. In contrast, theta decreased significantly during AW ($p<0.02$) compared to RC in only the scalp record. Generally, the state specific changes in theta activity compared to RC were accounted for by downshifts into the delta range at both sites. Furthermore, the scalp record during RC shows the expected relatively greater alpha activity, while AW shows significantly increased alpha activity ($p<0.02$) compared to RO in the depth record only. Differential theta activity recorded simultaneously at two different sites may offer some clues as to the functional changes that occur within specific brain regions according to behavioral state.

516.10

CROSS VALIDATION OF EVENT-RELATED POTENTIAL (ERP) MARKERS OF ADULT DYSLEXIA BY REGIONAL CEREBRAL BLOOD FLOW (rCBF). C.E. Naylor, M.R. Harter, F.B. Wood*, D.L. Flowers*, and I.S. Brown. Section of Neuropsychology, Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, NC 27103 and University of North Carolina-Greensboro, Greensboro, NC 27412

Previous studies (Harter et al. and Naylor et al.) showed a left central P240 deficit in dyslexic children and a bilateral central P240 deficit in dyslexic adults, respectively, in a visual letter discrimination task. Flowers, et al., showed focal left temporo-parietal rCBF increases in dyslexic adults, compared to controls, in an auditory word length analysis task.

In a sample containing 13 dyslexic and 10 non-dyslexic adults, both defined by childhood reading scores, the abnormal left temporo-parietal flow increase was correlated ($p < .01$) with both left and right central P240 as well as with left and right frontal P240 amplitude reductions. While this suggests convergence on a common deficit, across modalities and across physiological methods, it raises an important question of ERP source localization which is discussed by reference to illustrative MRI findings from individual subjects.

516.11

HYPERTENSION AND BODY POSITION AFFECT CAROTID ARTERY BLOOD FLOW VELOCITY DURING THE VALSALVA MANEUVER. I.E. Holden, B. Cimprich, B.L. Metzger*, B. Therrien. The University of Michigan, Ann Arbor, MI 48109

Persons with cerebrovascular disease are at risk from the marked shifts in blood flow evoked by the Valsalva maneuver (VM) or straining. We examined the influence of age, hypertension and position on carotid artery blood flow velocity (CABFV) during strain in the 70° upright and supine bed positions. Young healthy (n=180) and hypertensive (n=25) adults aged 30-55 years and older healthy subjects (n=66, >55 years) were studied. Subjects strained by blowing into a pressure gauge to 40 mmHg for 10 seconds. CABFV was measured by noninvasive Doppler technique. In the supine position, CABFV fell an average of 59% during strain for young hypertensives (p=.05). Following release of strain, young hypertensives had significantly greater overshoot above baseline than young healthy subjects (25% vs 17%, p=.01) and tended to have a greater overshoot than the older healthy group. During strain, supine and upright positions differentially altered CABFV in both young healthy subjects (-60% vs -46%, p<.05) and young hypertensives (-79% vs -56%, p=.001). Across the VM, total percent change in CABFV was less in the upright position vs supine for young hypertensives (699% vs 999%, p=.01) but not for controls. We conclude that CABFV changes during the VM are intensified in young hypertensives as compared to young and older healthy adults and that the intensity of these changes can be modified by body position.

*Supported by NIH, NCNR, grant #5 R01 NR01142-05.

516.12

COMPUTER-PROCESSED EEG FOR ANESTHETIC AND PSYCHIATRIC MANAGEMENT OF ELECTROCONVULSIVE SHOCK THERAPY (ECT). Michael R. Isley*, Paul G. Shinkman, Enid R. Kafer*, Dwight L. Evans, and Robert N. Golden*. Departments of Anesthesiology, Psychology, and Psychiatry, University of North Carolina, Chapel Hill, NC 27599.

Monitoring the brain during ECT has typically been restricted to the measurement of seizure duration, the most significant variable for therapeutic efficacy. With the introduction of "modified" ECT (i.e., the use of intravenous anesthetic drugs, neuromuscular blockade and controlled ventilation), this therapy now significantly involves anesthesiology. Thus, the purpose of the present study was to apply continuous monitoring of computer-processed EEG and EMG activity during ECT in order to tailor anesthetic and paralytic management to individual needs, and establish a reliable technique for on-line evaluation of seizure duration. All consenting patients met the DSM-III-R criteria for a major depressive episode (15 patients received 115 treatments). Nondominant unilateral or bilateral ECT was administered using brief bipolar pulses delivered by the Multiple-Monitor Electroconvulsive Therapy Apparatus (MECTA Corp., Portland, OR). Two channels of analog and computer-processed EEG were continuously monitored. The latter method involved power spectral analysis and display of the EEG as a dot-density modulated spectral array (DSA) with trended rms amplitude and spectral edge frequency (SEF 90%), using the 2-channel SRD Cerebro-Trac 2500+ (Misgav, Israel) and a standard ipsilateral, frontal-mastoid electrode configuration for the left and right hemispheres. The DSA profiles demonstrated that anesthetic induction, neuromuscular blockade, ECT seizure duration and recovery from each were more easily and consistently interpreted than using analog waveforms. We conclude that computer-assisted brain monitoring is superior to conventional techniques, and offers expanded capabilities for research on ECT and for its clinical management.

516.13

SCHIZOPHRENIA AND THE FRONTAL LOBES: COMPARISON OF MULTIPLE IMAGING TECHNIQUES AND NEUROPSYCHOLOGICAL TESTING. D.S. O'Leary, S.A. Kuperman*, G.A. Cohen*, and N.C. Andreasen NIMH-CRC, Psychiatry Department, University of Iowa, Iowa City, IA 52242.

Magnetic Resonance Imaging (MRI), Brain Electrical Activity Mapping (BEAM), and neuropsychological tests presumed to be mediated by the frontal lobes were administered to 41 patients with schizophrenia (n=35) or schizophrenia spectrum disorder (n=6), and to 23 normal controls. Regional Cerebral Blood Flow (RCBF) measures were also administered to 35 of the patients and 16 of the controls. BEAM and RCBF were conducted after a 21 day drug wash, or in 9 first episode patients, prior to neuroleptic treatment. MRI, BEAM, RCBF, and neuropsychological data were independently used to categorize patients into frontal lobe impaired and non-impaired groups. Two methods were used to dichotomize the group: a purely statistical method (i.e. upper quartile of each distribution is defined as impaired), and a more clinically-based technique (e.g. significant frontal slowing on BEAM, multiple perseverative errors on Wisconsin Card Sorting Test). Bayesian probabilities are used to compare the categorization produced by the two methods of dichotomizing the patients on each of the imaging and testing procedures. Results are complex and illustrate the difficulty of defining frontal lobe impairment in disorders such as schizophrenia in which there is no obvious lesion.

516.14

THE EFFECTS OF CONDITIONING MUSCULOCUTANEOUS STIMULATION ON THE SOLEUS H-REFLEX DURING WALKING IN SPASTIC PARETIC SUBJECTS. **J. Fung and H. Barbeau. School of Physical & Occupational Therapy, McGill University. Montreal, Quebec H3G 1Y5**

The soleus H-reflex amplitude in normal subjects is modulated throughout the gait cycle, which can further be inhibited by a conditioning musculocutaneous stimulation from the ipsilateral sole of the foot (Fung & Barbeau, Soc. Neurosci. Abstr. 15:1201, 1989). The present study was conducted to contrast the pattern of responses in 10 spastic spinal cord injured subjects during treadmill walking. The test soleus H-reflex was obtained by stimulating the tibial nerve in the popliteal fossa with a single 1 ms pulse, at an intensity that yielded a minimally detectable and constant M-response. The conditioning stimulus, consisting of an 11 ms train of three 1 ms pulses at 200 Hz, and preceding at 45 ms, was delivered to the ipsilateral medial plantar arch at a maximally tolerable intensity (up to 3X sensory threshold). The stimulation was delivered randomly in 8 different phases of the gait cycle.

Three different patterns of test responses could be identified. Two subjects showed a similar pattern to the normal, such that the H-reflex amplitude was reduced in early stance, progressively increased from midstance to push off, and was again decreased in swing. Four subjects showed generally elevated responses throughout stance, with a slight degree of depression present in swing. Four other severely spastic subjects showed a consistently elevated and similar H-reflex amplitude throughout the gait cycle with no phasic modulation. With musculocutaneous stimulation, the soleus H-reflex was significantly inhibited in the early stance and swing phases in 6 out of 8 subjects, but the trend was also present in the other 2 subjects. The effect was more pronounced in swing than in stance, relating functionally to an improved ability for the limb to swing through. The other two extreme cases included 1 mildly spastic subject who showed an almost complete inhibition of the H-reflex throughout stance as in the normal, and 1 severely spastic subject who showed an inhibition only in swing. The results support that Ia gating mechanisms are defective in spastic paretic gait, which can be modified by musculocutaneous stimulation. However, tonic gain mechanisms cannot be excluded. (Supported by the MRC)

516.15

RELIEF OF SPASTICITY BY TENS IS ASSOCIATED WITH IMPROVEMENT IN REFLEX AND VOLUNTARY MOTOR FUNCTIONS. C.W.Y.Chan and M.F.Levin. School of Physical and Occupational Therapy, McGill University, Montreal, Canada. H3G 1Y5.

Our previous studies showed that single, 45 min applications of transcutaneous electrical nerve stimulation (TENS) prolonged H and stretch reflex latencies in hemiparetic subjects. In addition, nine, daily 30 min TENS applications enhanced vibratory inhibition of the H reflex and tended to decrease hyperactive stretch reflexes. These findings suggested that longer term TENS may be effective in the reduction of hemiparetic spasticity. Our present objectives were two-fold: to determine whether longer-term TENS stimulation would lead to a reduction in clinical spasticity and whether such a reduction could be associated with a decrease in stretch reflex excitability and an improvement in voluntary motor function.

Thirteen spastic hemiparetic subjects participated in the study. The effects of fifteen, daily 60 min TENS treatments over a three week period were contrasted with those of placebo stimulation applied to the common peroneal nerve of the affected leg. The test battery consisted of five measures of reflex and voluntary function in the calf. They were 1) clinical spasticity scores, 2) maximal H reflex to M response ratios, 3) vibratory inhibition of the H reflex, 4) stretch reflexes, and 5) maximal voluntary isometric plantarflexion and dorsiflexion in standing.

In contrast to placebo stimulation which produced no significant effects, repeated applications of TENS decreased clinical spasticity ($p<0.05$), increased vibratory inhibition of the H reflex ($p=0.02$), and decreased the magnitude of stretch reflexes ($p=0.05$) in the spastic ankle extensors. These changes occurred concomitantly with a substantial improvement in voluntary *dorsiflexion* force ($p<0.05$) and a decrease in agonist/antagonist EMG co-contraction ratios ($p<0.05$).

Our results thus demonstrated that repeated applications of TENS can reduce clinical spasticity and improve control of reflex and motor function in hemiparetic subjects. Furthermore, the mechanism of the improvement may be partly related to an enhancement in presynaptic inhibition, and a possible 'disinhibition' of descending voluntary commands to flexor motoneurons.

516.16

SPASTICITY IS INVERSELY CORRELATED WITH ANTAGONIST VOLUNTARY CONTRACTION IN SPASTIC HEMIPARETIC SUBJECTS. M.F.Levin and C.W.Y.Chan. School of Physical and Occupational Therapy, McGill University, Montreal, Canada. H3G 1Y5.

The correlation between the severity of spasticity and residual voluntary muscular activity is unclear, yet the latter is often used to investigate the effects of treatment in spastic movement disorders. The objectives of our study were to compare voluntary EMG and force generated by ankle plantar- and dorsi-flexors in normal and spastic hemiparetic subjects, and to investigate the reproducibility of these measures and their correlation with clinical spasticity.

Seven age-matched normal subjects were tested once, and thirteen spastic hemiparetic subjects at least twice with one week apart. Subjects generated maximal isometric ankle plantar- and dorsi-flexion in the standing position. Agonist and antagonist EMG areas, co-contraction ratios, maximal force and temporal characteristics of force production were compared between affected and non-affected legs of hemiparetic subjects, after the latter were found to behave similar to legs of normal subjects. Spasticity was evaluated by clinical scales.

During dorsiflexion, maximal agonist EMG area and force were significantly decreased to 39% and 33% respectively of the non-affected leg. During plantarflexion, agonist EMG and force was reduced to 63% and 59% resepectively of the non-affected leg. Measures of maximal and mean force, force onset and dorsiflexion co-contraction ratios were highly reproducible ($r=0.78$ to 0.99), while raw and normalized EMG area measures were less so. Most interestingly, the amount of dorsiflexing force produced by the paretic dorsiflexors was highly correlated with the amount of agonist/antagonist co-contraction ($r=0.91$), and inversely related to clinical measure of antagonist spasticity ($r=-0.65$).

The high reproducibility of the force measurements suggested that they could be used to evaluate the effects of therapeutic intervention over time. More importantly, our findings demonstrated that, in hemiparetic subjects, the motor deficit in the paretic dorsiflexors but not the spastic plantarflexors was a reliable and valid indicator of the *severity* of ankle spasticity.

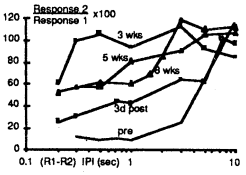
516.17

Long term recovery of sensory-motor function after spinal cord contusion in the rat. J.A. Gruner, N. Levine*. Dept. of Neurosurgery, NYU Medical Ctr., 550 First Ave., New York, NY 10016.

Following many types of CNS injuries, neurological function gradually recovers. Little is known, however, about the processes underlying this recovery. In spinal cord injury (SCI), the recovery time course is very consistent for physiological and behavioral measures; function is depressed for 1 wk, then gradually improves for another 2 to 3 weeks, and then plateaus.

To investigate the mechanisms of recovery after SCI, we developed a spinal cord contusion device capable of measuring the precise impact velocity and extent of cord contusion. Three neurophysiological tests were used to assess function after 12.5 or 25 mm x 10 gm weight drops: auditory startle responses (ASR), hindlimb myoelectric responses evoked by cerebellar stimulation (MEP), and somatosensory evoked potentials (SEP).

Rats were anesthetized and, using sterile surgical procedures, a T10 laminectomy performed to expose the spinal cord. The cord was contused and the wound closed. Evoked potentials were monitored before and after contusion. ASR, MEP, and SEP amplitudes showed a similar time course to that found previously. Of considerable interest, however, was the fact that MEP amplitudes, which typically begin to drop between 1 and 0.2 Hz, showed a substantial increase in ability to follow higher frequencies which was maintained as long as 8 weeks after injury (see figure).



ALZHEIMER'S DISEASE: NEUROPATHOLOGY III

517.1

REGIONAL DIFFERENCES IN CORTICOTROPIN-RELEASING FACTOR (CRF) AND SOMATOSTATIN (SRIF) IN ALZHEIMER'S DISEASE. W. H. Smith, G. Bissette, L. Cook, B. Crain, K. Dole and C.B. Nemeroff. Depts. of Psychiatry, Pharmacology and Pathology, Duke Univ. Med. Ctr., Durham, NC 27710.

Although several neuropeptides have been implicated in the pathology of Alzheimer's disease (AD), only corticotropin-releasing factor (CRF) and somatostatin (SRIF) have consistently been found to be reduced in post-mortem brain tissue in AD. The present study measured these two neuropeptides in 38 discrete brain regions. Tissue from 10 controls which consisted of both demented (non-AD) and non-demented patients, were compared to tissue from 15 patients with neuropathologically confirmed AD. CRF and SRIF concentrations were determined by sensitive and specific RIAs. CRF concentrations in AD patients were significantly lowered in 11 cortical regions (Brodmann's areas 4, 7, 10, 11, 12, 20, 21, 22, 39, 44 and middle frontal gyrus), the amygdala, insula, hypothalamus, and putamen. The concentration of SRIF was significantly decreased in 8 cortical regions (Brodmann's areas 6, 12, 17, 20, 21, 24, 39, 40), and the insula. These results differ somewhat from previous studies in the pattern of neuropeptide alterations. Such interstudy variations may reflect unique neuropathological changes manifested by individual disease course or etiology. Supported by NIMH MH-40524 and NIA AG-05128.

517.2

ALZ-50 IMMUNOREACTIVE PROFILES IN HIPPOCAMPAL COMPLEX OF AGED AND ALZHEIMER'S DISEASED (AD) BRAIN. D.R. Brady and E.J. Mufson. Inst. Biogeront. Res., Sun City, AZ 85351.

Alz-50 is a putative marker for cytoskeletal degeneration in AD. However, virtually no detailed studies exist that compare Alz-50 profiles in normal aged and AD brains. We used the Alz-50 antibody to ascertain the morphology and topography of Alz-50 immunoreactive (IR) profiles in postmortem paraformaldehyde fixed hippocampal formation (HF) and entorhinal cortex (EC) from "normal aged" ($\bar{X}$=73 yrs; n=10), "pre-senile" (clinically normal with moderate AD pathology; $\bar{X}$=85.5 yrs; n=4) and AD ($\bar{X}$=80 yrs; n=3) brains. Alz-50 staining in normal and pre-senile brains revealed Golgi-like neurons in CA2, CA1-Subiculum (Sub) and EC. In four normal and all pre-senile cases many dentate granule cells were also IR. Six normal and all pre-senile cases showed IR particulate in the neuropil of the dentate molecular layer, strata moleculare and oriens of CA1 and CA2, Sub and EC. In contrast, Alz-50 staining in AD showed numerous dystrophic neurons with marked neurofibrillary tangle (NFT)-like morphology throughout the HF and EC. In AD, dense Alz-50 IR neuropil staining differentiated each hippocampal subfield. Compared to HF and EC of normal brain, quantitation revealed 68% and 90% more IR neurons in pre-senile and AD, respectively. Sections stained with anti-ubiquitin (Ub) revealed a few IR NFTs in HF and EC of normals. In pre-senile cases, a small population of Ub IR NFTs were seen within CA1-Sub and layer 2 of EC, compared to numerous IR NFTs throughout HF and EC in AD cases. Thioflavin-S staining in normal aged HF and EC revealed a few random NFTs, whereas pre-senile cases contained many more NFTs with a preponderance in EC (68%). When present in normal and pre-senile cases, Alz-50/thioflavin-S double labeled profiles occupied CA1 and layer 2 of EC. In AD brains, thioflavin-S NFTs were 13 times more prevalent than in pre-senile brains, occupying CA1-4, Sub and EC. Many of these NFTs were also double labeled with Alz-50. Since a significant population of HF and EC neurons express Alz-50 in normal aged and pre-senile brains, it is possible that Alz-50 expression is a consequence of normal aging which is exacerbated in AD. Support: ADCRC 82-0686, YK-8365 and AHAF.

517.3

A LINE OF PLAQUES IN AREA 17 OF ALZHEIMER'S DISEASE: IMPLICATIONS FOR PATHOGENESIS. T.G. Beach and E.G. McGeer. Kinsmen Laboratory of Neurological Research, Univ. of British Columbia, Vancouver, B.C. Canada.

The laminar distribution of staining for senile plaques in area 17 was investigated in 16 patients with Alzheimer's disease (AD) and compared to the normal laminar distribution of afferent fibres, vascularity, acetylcholinesterase (AChE), and other chemoarchitectonic features. Senile plaques were aggregated at significantly higher density at the interface of laminae IVc and V. This did not correspond with any reported afferent, chemoarchitectonic, or vascular distribution. The line of plaques observed at the IVc/V interface is similar to that observed in the dentate gyrus (Crain, B.J., et al, Acta Neuropathol., 76:87, 1988). The line of plaques in the dentate gyrus also has no apparent correlate in normal anatomy, but, as in area 17, is located at an interface between laminae. At both sites, the line of plaques form where an Alz-50-immunoreactive neuritic field (Hyman, B.T., et al, Brain Res., 450:392, 1988; Beach, T.G., et al, Brain Res., 501:171, 1989) marking degenerating neurites meets a layer which has a high capillary density and a high cholinergic terminal density. The interaction between one or more of these elements may therefore be contributive to senile plaque formation.

517.4

STABILITY OF SYNAPSE NUMBERS IN THE ENTORHINAL CORTEX (AREA 28) IN PATIENTS WITH ALZHEIMER'S DISEASE. S.W. Scheff and D. A. Price*. Center on Aging, Dept. Anatomy & Neurobiology, Univ. Kentucky, Lexington, KY 40536-0230.

The parahippocampal gyrus, a portion of the temporal lobe and key component of the limbic system, lies adjacent to the hippocampus. Both structures are closely linked with human memory and known to be affected in Alzheimer's disease (AD). One part of this gyrus is the entorhinal cortex which receives information from other limbic structures and transmits it to the hippocampal formation. We have previously shown that several cortical regions (superior frontal, superior and middle temporal) demonstrate a highly significant reduction in synaptic density in AD. Because of the extensive connections of the entorhinal cortex with other limbic structures and its known involvement in AD, it is important to quantitatively assess its functional connectivity (synaptic density) and to determine the degree of neuropathological changes in AD.

Human brains were obtained at postmortem examination from 10 patients who met the NINCDS-NIA criteria for AD and from 10 age-matched controls. All tissues were obtained with 13 hours postmortem. Both lamina III and V were quantitatively assessed for synapse number and size. Unlike the widespread and significant loss of synapses in some association cortex, the entorhinal cortex shows ***no apparent synaptic decline***. This finding might be the result of a compensatory mechanism which overcomes the possible loss of connectivity as a result of cell loss in brain regions known to project to the entorhinal cortex. Supported by Alzheimer Association IIRG 87-042 and NIA AG05144.

517.5

AMYGDALAR DENDRITIC REGRESSION IN ALZHEIMER'S DISEASE: QUANTITATIVE GOLGI ANALYSIS. S.A. Scott, D.L. Sparks, S.T. DeKosky and S.W. Scheff. Sanders-Brown Center on Aging and Dept. Anatomy & Neurobiology, Univ. of Kentucky, Lexington, KY 40536-0230 and Depts. Psychiatry and Neurology, Univ. Pittsburgh Sch. of Med., Pittsburgh, PA 15261.

The amygdala undergoes severe degeneration in Alzheimer's disease (AD). To ascertain its available substrate for synaptic connectivity, we used a Golgi-Kopsch modification to study dendrites of the magnocellular basal amygdaloid nucleus (BNMC). The BNMC receives an especially dense projection from nucleus basalis of Meynert.

Five cognitively normal aged controls were compared to 4 AD cases (postmortem interval less than 5hr) meeting NINCDS-NIA criteria. Employing 15 neurons per case, the dendritic arbor was ordered somatofugally and each segment measured using an interactive, on-line computer tracing program (Kontron IPS). Mean total dendritic length per neuron was ***reduced*** in AD by 22%, due largely to changes among intermediate order segments. Significant alterations in total number of segments and average segment length, however, were not found. Despite the overall surface loss, ***increases*** in dendritic length and number with AD were noted amongst the ***most distal*** segments. These data suggest the presence of a subpopulation of excessively branched neurons in AD. However, since the majority have regressed dendritic trees, the net result is atrophy, suggesting a reduced amygdalopetal circuitry with key CNS regions.

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517.6

UBIQUITIN IMMUNOREACTIVE DYSTROPHIC NEURITES IN SELECT AREAS OF THE NEOCORTEX IN DOWN'S SYNDROME. L.A. Mattiace, Y. Kress*, P. Davies*, S-H Yen and D.W. Dickson.
Dept of Pathology Albert Einstein College of Medicine, Bronx, NY, 10461.

Ubiquitin immunoreactive structures were studied in Down's syndrome brains ranging in age from 2 days to 60 years. Numerous randomly distributed ubiquitin immunoreactive dot-like structures in the white matter were shown to correspond to granular degeneration of myelin. These structures were first detected at age 21 and increased progressively thereafter with age. Other larger and more coarsely granular ubiquitin immunoreactive structures, most numerous in the middle and upper cortical layers, were consistent with dystrophic neurites. These ubiquitin immunoreactive structures were first detected at age six in the hippocampus and appeared to increase progressively with age. Immunoelectron microscopy demonstrated that these dystrophic neurites did not contain filamentous material. In the presence of amyloid in Down's syndrome adults, there was a substantial increase in the number of ubiquitin immunoreactive structures in the grey matter, beyond the increases seen with age. In the presence of diffuse amyloid plaques, these ubiquitin immunoreactive neurites were randomly distributed throughout the neuropil. However, in the presence of compact amyloid deposits, these ubiquitin immunoreactive dystrophic neurites formed plaque-like aggregations. This suggests that the disruption of the neuropil in areas of amyloid deposition may promote the formation of dystrophic neurites. In addition, since the presence of these dystrophic neurites appeared substantially earlier in the grey matter in Down's syndrome than in age-matched normals, this may be further evidence that selective aspects of aging may be accelerated in Down's syndrome individuals.

517.7

QUANTITATIVE NEUROPATHOLOGIC ANALYSIS OF ALS-PD CASES FROM GUAM. P.R. Hof, D.P. Perl, J.C. Steele[1]*, W. Janssen and J.H. Morrison. Fishberg Res Ctr and Dept of Geriatrics, Mt Sinai Sch Med, New York, NY 10029, and [1]Guam Memorial Hospital, Guam.

Guam, one of the Marianna islands in the Western Pacific, is characterized by a very high prevalence of ALS and Parkinson-Dementia (PD) in certain villages. We have undertaken a quantitative analysis of the laminar and regional distribution of neurofibrillary tangles (NFT) in the cerebral cortex of several cases presenting with different clinical symptomatology. Preliminary results revealed that areas within the temporal lobe are more heavily affected than frontal or parietal regions in PD cases. In particular, the hippocampal formation contained the highest NFT densities (up to 200 NFT/mm cortical traverse). ALS cases had very few cortical lesions. There were striking differences with Alzheimer's disease (AD) cases: NFT in neocortical association areas of Guam cases always predominated in superficial layers (up to 3-times more NFT in layer III than in layer V), whereas in AD, NFT are more frequently observed in layer V. The motor cortex was consistently involved in Guam cases, while it has very few NFT in AD. No amyloid was observed in any of these Guam cases. The differential NFT distribution in Guam cases still supports the hypothesis that a global corticocortical disconnection occurs in dementia, but suggests that in these cases a more circumscribed set of projections may be involved than in AD. These data are consistent with the notion that the disease may be caused by a pathogen retrogradely transported along specific corticocortical circuits that project heavily to mediotemporal areas. Supported by grants from NIH AG06647 and AG08802.

517.8

DOWN'S SYNDROME (DS), DEMENTIA PUGILISTICA (DP), AND ALZHEIMER'S DISEASE (AD): A QUANTITATIVE NEUROPATHOLOGIC COMPARISON. C. Bouras[1], P.R. Hof, R. Guntern[1]*, and J.H. Morrison. Fishberg Res Ctr and Dept of Geriatrics, Mt Sinai Sch Med, New York, NY 10029, and [1]Dept of Psychiatry, Univ of Geneva Sch Med, Geneva, Switzerland.

There is evidence that dementia in AD results from the global disconnection of specific corticocortical pathways. In order to further test this hypothesis we compared the laminar and regional distribution of neurofibrillary tangles (NFT) and neuritic plaques (NP) in 5 DS and 1 DP cases with brains from AD patients. The distribution of NFT and NP in DS was qualitatively and quantitatively comparable to that observed in AD, however lesions were found only in DS cases over 40 years. In association cortex NFT were predominant in layer V, whereas NP were more numerous in III. DS patients over 50-year old showed more lesions than cases below 50. The brains of two infants with DS had no lesions. In contrast, to the lesion distribution observed in AD and DS, the brain from a 58-year old retired boxer showed a massive involvement of the superficial layers: very high NFT densities were encountered in layer III throughout the cerebral cortex, while very few NFT were observed in V. No amyloid deposits or NP were present in the DP case. It is interesting to note that NFT predominate in layer III in both DP and Parkinson-Dementia of Guam, but are more equally distributed across III *and* V in DS and AD. This suggests that both DS and AD may involve a very global disruption of corticocortical circuits, whereas a subset, such as feedforward circuits may be more selectively involved in DP and Parkinson-Dementia of Guam. Supported by NIH grant AG06647 and ADRC AG05138.

517.9

NEUROPATHOLOGIC CHANGES IN THE HIPPOCAMPAL FORMATION OF ALZHEIMER'S DISEASE PATIENTS: A QUANTITATIVE MRI ANALYSIS. S.J. Kirsch, R.W. Jacobs, L.L. Butcher, & J. Beatty. Behavioral Neuroscience Program, Department of Psychology, UCLA, Los Angeles, CA 90024.

Traditionally, the monitoring and diagnosis of a neurologic disorder using magnetic resonance imaging (MRI) was based solely on the visual images obtained. New low field (LF) MRIs, however, greatly increase T1 and T2 tissue contrasts (which reflect the molecular environment of the tissue), allowing for easy and reliable quantification of different, or changing, tissues. Exploiting this advantage, T1 and T2 relaxation times of the hippocampal formation (HF) were measured in 21 normals (age 18-79 yrs) and 4 patients with suspected Alzheimer's disease (AD)(age 69-81yrs) using an Instrumentarium Magnaview 0.04T LF MRI (T1 = IR:1500 (50,375)/40; T2 = SE:1000/130 and 1000/200). A mid-thalamic, 10-mm coronal slice through the HF revealed T1 times of ADs to slightly, but consistently, exceed those of normals. T2 times of ADs, however, were substantially lengthened (by 15-65ms), and appear to be related to the degree of dementia. In addition, there were indications of differential pace of AD pathology in the two hemispheres. These results suggest that LF MRI may provide a means for diagnosing and quantifying the pathology of AD.

517.10

ENTORHINAL CORTEX IS AN EARLY SITE OF NEUROFIBRILLARY TANGLE PATHOLOGY IN NON-DEMENTED ELDERLY. P.V.Arriagada, B.T.Hyman. Dept. Neuropathology & Neurology, Massachusetts General Hosp, Harvard Medical School, Boston, MA 02114.

It is known that the occurrence of Senile Plaques(SP) and Neurofibrillary tangles (NFT) seems to increase with age in nondemented elderly. We have studied the question of whether certain cytoarchitectural areas are consistently vulnerable, and whether there is a relationship between the appearance of NFT and SP with one another and with age. We examined 15 cytoarchitectural areas including the hippocampal formation, entorhinal cortex (EC), amygdala, nucleus basalis, and neocortical areas 20 and 21 in 20 cases of presumed non-demented elderly, ages 58-85 years, using ThioflavinS (Thio S) and Alz-50 immunocytochemistry . Quantitation of NFT (Thio-S) showed that the greatest severity of involvement was in the EC . The severity of NFT pathology in the EC increased exponentially with age ($R=.600$, $p<.005$). The number of NFT in other areas (combined) was significantly correlated with the number of NFT present in the EC (Spearman correlation, $p<.004$). Although amyloid SP were present in nearly all cases, Alz-50 positive SP and immunoreactive dystrophic neurites were found only in cases with substantial NFT (more than 35 NFT/1.8 mm2 field) in EC. These results suggest that NFT and SP accumulate in a stereotyped, hierarchical fashion with age,and that the EC is an early site for NFT pathology . In addition, the presence of Alz-50 positive SP and dystrophic neurites is related to the severity of NFT. (Supported by the Brookdale Foundation, the Educational Commission for Foreign Medical Graduates and NIH AG08487). We thank P.Davies,New York, for the gift of Alz-50).

EPILEPSY: KINDLING II

518.1

TRANSFER KINDLING FROM THE AMYGDALA OR PIRIFORM CORTEX TO THE FRONTAL CORTEX IN FEMALE RATS WITH AND WITHOUT ESTRADIOL REPLACEMENT. G. G. Buterbaugh and G. M. Hudson*. Department of Pharmacology and Toxicology, University of Maryland School of Pharmacy, Baltimore, MD 21201.

In ovariectomized (OVX) female rats, estradiol (E) replacement facilitates kindling of the amygdala (Am) and frontal cortex (fCtx), but not the piriform cortex (pCtx). We therefore tested the influence of E on secondary fCtx kindling following antecedent kindling of E-sensitive (Am) and E-insensitive (pCtx) primary sites. Ten days after OVX, rats were kindled by daily Am or pCtx stimulation. At least two weeks later, daily fCtx kindling stimulations were initiated in these rats, with (E) and without (nE) estradiol replacement. Am-kindled nE rats completed secondary fCtx kindling after 13.6 $\pm$ 2.3 afterdischarges (AD) and 559 $\pm$ 132 AD sec, and pCtx-kindled nE rats completed fCtx kindling after 13.5 $\pm$ 2.4 ADs and 374 $\pm$ 51 AD sec, significant savings compared to fCtx primary site kindling of nE rats (38 $\pm$ 2.2 ADs and 840 $\pm$ 93 AD sec). In Am-kindled rats, E replacement did not significantly effect secondary fCtx kindling. In pCtx-kindled rats, E rats completed secondary fCtx kindling after significantly fewer ADs (32%) and AD sec (44%), compared to nE rats. These results suggest that E facilitates the kindling process by interacting with an anatomical/mechanistic substrate shared by E-sensitive kindling sites. (Supported by PHS NS20670)

518.2

PERFORANT PATH EVOKED SYNAPTIC CURRENTS IN THE DENTATE GYRUS STUDIED WITH CURRENT SOURCE DENSITY TECHNIQUES AFTER LTP AND KINDLING G. Golarai, T. Sutula. Neuroscience Training Program, Depts. of Neurology and Anatomy, University of Wisconsin, Madison, WI 53792.

The perforant path demonstrates physiological and morphological plasticity including reversible increases in synaptic efficacy (LTP) and kindling-induced synaptic reorganization. CSD analysis was used to investigate functional consequences of these phenomena in dentate gyrus (DG). CSD analysis in normal rats revealed the location of currents underlying PP evoked field potentials in the DG. LTP increased the magnitude but did not alter the spatial pattern of PP evoked currents in the DG. In contrast, PP activation in kindled rats evoked an altered pattern of current flow including a net inward current in the inner molecular layer at 5-7 msec after granule cell discharge. The spatial location of this current corresponds to the terminal zone of sprouted mossy fibers, suggesting that axonal sprouting and synaptic reorganization of the mossy fiber pathway may have functional consequences that could include increases in recurrent excitation mediated by the sprouted collaterals.

518.3

DEVELOPMENT OF POSTICTAL EEG DEPRESSION IN AMYGDALA-KINDLED RATS. B. M. Pedrosi* & R. F. Berman. Dept. Psychology, Wayne State University, Detroit, MI 48202.

Seizures produced by the kindling process are usually followed by a period of flattened electroencephalographic (EEG) activity called postictal EEG depression. The development of this phenomenon was observed in treatment-free subjects. Twelve, male, Long Evans rats were stereotaxically implanted with electrodes into the medial amygdala. Electrical brain stimulation thresholds that produce a brief epileptiform afterdischarge were then established for each rat. Animals were stimulated at threshold until five consecutive Stage 5 or 6 behavioral seizures were attained. Observations were made of the development of the afterdischarge and the duration of postictal EEG depression. The development of postictal spiking was also recorded. Postictal EEG depression appeared as early as Stage 0, and was represented in all subsequent stages. In addition, the duration of postictal EEG depression increased over the course of kindling, suggesting that inhibition also "kindled" over repeated stimulation trials. However,correlations between the durations of afterdischarge and postictal EEG depression were uniformly low and not statistically significant ($r^2=0.12$) indicating that the underlying mechanisms responsible for these two phenomena are different. Thus, kindling appears to be associated with long-lasting changes in both excitatory and inhibitory neural processes. (Supported by Grant No. RR-08167)

518.4

AMYGDALA KINDLING INFLUENCES THE EXCITABILITY OF BRAINSTEM SUBSTRATES. C.D. Applegate, G.M. Samoriski* and J.L. Burchfiel. Comprehensive Epilepsy Program, University of Rochester School of Medicine, Rochester, NY 14642.

Previous research has demonstrated a role for pontine reticular structures in the expression of tonic hindlimb extension (THE) following ECS stimulation (Browning, Fed. Proc., 44:2425, 1985). This study investigated the influence of amygdala kindling on ECS-induced THE. Male, Sprague-Dawley rats were implanted with bipolar electrodes into the left amygdala (N=14). Following recovery, the incidence of THE to a corneal ECS-stimulus was determined. Rats were then kindled to 5 consecutive stage 5 seizures, and the incidence of THE was again determined. In comparison with unkindled control animals (N=23), kindling significantly increased the incidence of THE from 23% to 79%. The incidence of THE in controls was 26% and 39% for test and retest, respectively. In a second experiment, amygdala-kindled rats (N=7) received mechanical lesions of the pontine reticular formation. While lesions blocked the expression of THE in these animals, no significant alteration in kindled seizure expression was observed. Results suggest that while kindling interacts with brainstem substrates necessary for aspects of tonic seizures, these same substrates are not necessary for kindled seizure expression.

518.5

c-fos Expression in the Magnocellular Neuroendocrine System During Amygdala Kindling

R.S. Greenwood, R.B. Meeker L. Rietz* and J.N. Hayward. Dept. of Neurology and Pediatrics and Neurobiology Curriculum, University of North Carolina, Chapel Hill, NC 27599.

In previous studies we have shown that resting plasma vasopressin (VP) is elevated and that vasopressin mRNA expression is chronically higher in the magnocellular neuroendocrine system after amygdala kindling. Since c-fos expression is elevated in other areas of the nervous system after seizures and may be involved in regulation of VP gene transcription we measured c-fos changes during the early phases of amygdala kindling. A 48 mer oligonucleotide specific for c-fos was 3'-end labeled with ^{125}I-dCTP and hybridized to 20 um sections from the brains of kindled or sham-stimulated rats. The hybridization of the antisense c-fos oligonucleotide probe was compared to hybridization of a sense oligonucleotide probe and to a specific probe for VP mRNA. Sections were analyzed with an image analysis system (Bioquant IV). A single train of the kindling stimulus (1 s, 60 cps, 1 ms, biphasic, 400 uA peak-to-peak trains) was administered to the kindled animals. A 50 % increase in c-fos mRNA without a corresponding change in VP mRNA expression was found. The c-fos mRNA in the cortex on the side of amygdala stimulation were also higher than in sham stimulated animals.

The results of this study suggests that proto-oncogenes could be involved in the regulation of vasopressin gene expression in kindled rats.

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518.6

AMYGDALOID KINDLING INDUCES TRH mRNA IN SPECIFIC LIMBIC FOCI AS DETERMINED BY IN SITU HYBRIDIZATION HISTOCHEMISTRY (ISHH). M.J. Kubek, S.M. Knoblach, K.S. Fuson and M.R. Aydelotte*. Depts. of Anatomy, Psychiatry, & Program in Medical Neurobiology, Indiana University & VA Medical Centers, Indianapolis, IN 46202.

TRH levels are increased after either electrical or chemical kindling (Ann.N.Y.Acad.Sci.553:286,1989). ISHH was used to study the possibility that TRH mRNA might change in specific seizure foci as a consequence of this process. Rats were either electrically (5 stage 5 seizures) or sham kindled. After 2 weeks rats were killed 1,3,6,12,& 24hrs following one stage-5 seizure. Sections were hybridized with a 35S-CTP labeled 548b TRH riboprobe. Film and emulsion were evaluated via computer image analysis. Significant TRH mRNA increases (% of sham) were seen in dorsal & ventral dentate gyri at 3hrs (658 & 96) & 6hrs (2098 & 596), peaked at 12hrs (2692 & 955), and returned to 3hr levels at 24hrs (510 & 138). Significant TRH mRNA increases were also measured in CA3, CA1, & piriform ctx at 3hrs and remained elevated at 24hrs. No hybridization was seen in these same regions in sham or 1hr groups. This report is the first to quantitate temporal changes of TRH mRNA in seizure foci following kindling. Normally, TRH mRNA is not detectable in these same regions. ECS has similar effects on TRH mRNA (this meeting). We suggest that TRH increases following kindling and ECS are the result of de novo synthesis in specific loci. Supported by NS25661.

518.7

THE EFFECT OF A 12-WEEK INTERVAL ON PERMANENCE OF 'PARTIAL' (STAGE 1), 'FULL' (STAGE 5) AND LOW FREQUENCY (3 HZ) KINDLING IN THE RAT. Z. Dennison, T. Tandan*, G.C. Teskey and D.P. Cain. Dept. of Psychology, Univ. of Western Ontario, London, CANADA N6A 5C2.

We previously compared the effect of a 12-week interval on kindling to stage 3 or stage 5 and found that both groups demonstrated equal permanence and fallback (S.N. Abstr. 1989, 310.9). Here we examined the permanence of partial (stage 1) 60 HZ kindling and full (stage 5) low frequency kindling. Rats with amygdala electrodes were kindled using 1-sec trains of 60 Hz pulses to stage 1 (ST1, 4 afterdischarges [ADs]) or stage 5 (CONT), or to stage 5 using 3 Hz pulses (LF). A second control group had AD threshold only determined (ADT). Rekindling after 12 weeks was performed using 1-sec 60 Hz trains in all rats. Results showed that ST1 and CONT required the same total no. of ADs to kindle to stage 5; therefore ST1 demonstrated significant savings over the interval. LF and CONT required the same no. of ADs to rekindle to the second stage 5; therefore LF kindling demonstrated similar permanence to 60 Hz kindling. Supported by an NSERC grant to DPC.

518.8

LOCALIZED CHANGES IN CYTOCHROME OXIDASE STAINING IN HIPPOCAMPUS OF KINDLED RATS. J.N. Nobrega, J. Petrasek, L.M. Dixon, S.J. Kish and W.M. Burnham, Clarke Institute of Psychiatry and Pharmacology Dept., Univ. of Toronto, Toronto, Ont., M5T 1R8, Canada.

Kindling of limbic structures has been associated with a number of alterations in hippocampus, including abnormal neuronal excitability, changes in Ca++ concentrations, increases in paired-pulse inhibition, and increased GABA-BZP binding. In the present study cytochrome oxidase (CO) histochemistry was used to probe possible sustained alterations in metabolic activity in the hippocampus of kindled rats. Relative CO activity, the terminal complex of the mitochondrial respiratory chain, is thought to reflect local tissue metabolic capacity, which is coupled to neuronal activity (Wong-Riley, M.T.T. *TINS*, 1989, 12, 94-101). Rats were kindled via stimulation of the entorhinal cortex and sacrificed either 24 hr (n=7) or 28 days (n=7) after their sixth stage-5 seizure, together with equal numbers of non-stimulated controls. Brains were processed for CO histochemistry using a modification of the Wong-Riley (1979) method. Ten hippocampal areas were subjected to densitometric analyses, using callosum optical density (OD) as an internal densitometric standard for each section. OD values were further normalized by the overall mean. Results for the 24 hr groups revealed a small (-4.3%), but highly reliable decrease in CO staining in the superior blade of the dentate gyrus of kindled rats, as well as 2-3% increases in the oriens layer of CA1 and the hilar region (all $ps <$ 0.001). After 28 days, these differences were slightly reduced and were no longer statistically significant. The observed changes may relate to previously reported acute alterations in hippocampal function, although their significance for the permanent kindled state is unclear. These preliminary results suggest that CO histochemistry may provide a sensitive method for assessing localized changes in kindled brains.

518.9

ICTAL AND INTERICTAL BRAIN EEG MAPPING DURING AMYGDALOID KINDLING IN THE CAT: POWER SPECTRAL AND TIME DOMAIN STUDY. A.Fernández-Guardiola, A.Martínez*, R.Fernández-Mas*, R.Gutiérrez. Neurosci. Div. Instituto Mexicano de Psiquiatría. México, D.F., 14370.

The pattern of the cortical propagation of amygdaloid afterdischarges (AM/AD), was studied in daily kindled cats. By means of a computer program, 4-second samples of the recordings with an isometric 16-channel cortical matrix were acquired a minute before and 2, 14, 26 and 38 sec after the amygdaloid stimuli during the process. Behavioral video tape recordings were simultaneously carried out. Power spectra (4-16 Hz) and historical layout of isolated AM/AD ictal and interictal spikes were computed during each behavioral stage (I-VI). The cortical projection of the amygdaloid activation throughout the process is different when ictal or interictal spikes are considered. The analysis of the interictal activity showed less spectral density than that of the AM/AD and the projection corresponded to the prefrontal and frontal ipsilateral cortices, the anterior portion of the ipsilateral temporal lobe and a restricted area of the contralateral temporal lobe. The AM/AD projection was more evident in the insular and ectosylvian posterior cortices, bilaterally in the frontal regions and a strong activation of the contralateral temporal lobe appeared towards the end of the AM/AD. The electrical and the behavioral manifestations, e.g., the direction of circling, mioclonia, and the preictal rigidity, are asymmetrical, even during stage VI. The average of the interictal EEG maps were clearly different for each stage and showed a progressive activation beginning in the frontal cortex, and latter appearing in the temporal lobe, contralateral to the stimulated amygdala. Experiments on kindled seizures are commonly considered as a model of Grand mal epilepsy, from our results we conclude that kindling is rather a model of temporal lobe partial complex epilepsy.

518.10

FUNCTIONAL AND METABOLIC EVIDENCE FOR UNILATERAL MEDIATION OF INFERIOR COLLICULAR CORTICAL SEIZURES. T.J. McCown, G.E. Duncan and G.R. Breese. University of North Carolina, Chapel Hill, N.C. 27599-2750.

Unilateral electrical stimulation of a specific area in the inferior collicular cortex produces bilateral behavioral seizures, which progress from wild running seizures after acute stimulation to generalized seizure behaviors after repeated "kindling" stimulation (McCown et al., Exp. Neurol. 86:527,1984). Initially, we found that unilateral stimulation initiated non-synchronous, bilateral afterdischarge in the inferior collicular cortex that coincided with the behavioral seizure activity. However, unilateral kindling in the inferior collicular cortex did not influence the kindling rate from the contralateral inferior collicular cortex. Furthermore, contralateral collicular cortical lesions did not alter ipsilateral seizure parameters. Using ^{14}C-2-deoxyglucose autoradiography, the unilateral nature of seizure activity in the inferior collicular cortex was substantiated. Thus, unilateral activity in the inferior collicular cortex is sufficient to cause bilateral seizure behaviors. (Supported by HD-03110 and NS-26595).

518.11

OPPOSITE CHANGES OF DOPAMINE TURNOVER IN THE PREFRONTAL CORTEX AND NUCLEUS ACCUMBENS AFTER AMYGDALOID KINDLING. P. Rada*, E. Murzi* and L. Hernandez. Laboratorio de Fisiologia and Unidad de Neurologia, Facultad de Medicina, Universidad de Los Andes, Merida, 5101-A, Venezuela

An antagonistic relationship between psychosis and epilepsy called "forced normalization" has been described[1]. When anticonvulsant administration suppresses seizures, psychotic episodes appear, and vice versa, when neuroleptic administration ameliorates psychotic symptoms, seizures occur more often. It is believed that seizure and dopamine (DA) turnover are related. In fact, DA receptor blockers lower threshold seizures in rats[2]. However, in brain homogenates, DA turnover changes have not been observed after amygdaloid kindling[3]. Microdialysis was used to monitor extracellular DA and its metabolites in the nucleus accumbens (NAC) and the prefrontal cortex (PFC) in amygdaloid kindled rats. Under ketamine anesthesia, 10 male Wistar rats were chronically implanted with ipsilateral monopolar electrodes in the amygdala for electrical stimulation; guide shafts for microdialysis were aimed to the NAC in 5 rats and to the PFC in the other 5 rats. After a week of recovery, animals were stimulated daily in the amygdala until three consecutive stage 5 motor seizures were elicited (square-wave pulses of 300 μA, 0.4 msec and 60 Hz). DA and its metabolites were assessed by HPLC with electrochemical detection. DA turnover decreased in the NAC and increased in the PFC with kindling. These opposite variations in extracellular DA as a result of kindling suggest dopaminergic mesolimbic and mesocortical involvement in epilepsy and a mechanism for the "forced normalization" phenomenon.

[1]Pakalnis, A., et al., *Arch. Neurol.*, 1987, 44: 289.
[2]Toshio, M., et al., *Psychopharmacology*, 1988, 94: 259.
[3]Blackwood, D. In P. L. Morselli et al. (Eds.), Raven Press: NY, 1981.

ALZHEIMER'S DISEASE: MOLECULAR STUDIES

519.1

EXPRESSION OF SULFATED GLYCOPROTEIN-2, THE RODENT HOMOLOGUE OF pADHC-9, INCREASES IN RAT HIPPOCAMPUS AFTER EXCITOTOXIC LESIONING. P. C. May and M. Lampert-Etchells*#. Lilly Research Labs, Eli Lilly and Company, Indianapolis, IN 46285 and Andrus Gerontol Ctr.#, USC, Los Angeles, CA 90089.

pADHC-9 is a hippocampal cDNA clone which is overexpressed in Alzheimer's disease hippocampus (May et al., 1989, Can J Neurol Sci. 16:473). Sulfated glycoprotein-2 (SGP-2), the rodent homologue of pADHC-9, is a major secretory product of the rat Sertoli cell and presumably is involved in male reproductive function; its role in brain is unknown. To explore possible functions, young male F344 rats were subjected to bilateral intraventricular injections of kainic acid (0.5 ug/ul each) and sacrificed 14 d later. Total RNA or protein was extracted from the dorsal hippocampi and used in RNA or immunoblot analyses. SGP-2 RNA levels increased 2 fold in KA-lesioned hippocampi compared to saline-injected controls ($p< 0.05$; n=3-4). As expected, GFAP RNA prevalence in hippocampus also increased 4 fold following KA lesioning ($p<0.02$; n=3-4). Immunoblot analyses of hippocampal homogenates revealed comparable increases in SGP-2 and GFAP protein expression following the lesion. Immunocytochemical analyses detected little SGP-2 immunoreactivity in non-lesioned animals but marked accumulation of SGP-2 in atrophic CA3 and CA4 pyramidal cells present 14 d after the lesion. These data indicate that the increased expression of pADHC-9 in AD hippocampus can be duplicated in the kainate-lesioned rat and this model will be useful for identifying the function of pADHC-9/SGP-2 in the brain. (Supported in part by grants to PCM from the ADRDA IIRG-88-069 and AFAR.

519.2

BASAL AND WATER SOLUBLE FORSKOLIN STIMULATED SECOND MESSENGER FORMATION (cAMP) IN POST MORTEM OLFACTORY BULB, HIPPOCAMPUS AND CEREBELLUM OF CONTROLS AND ALZHEIMER-PATIENTS. T.G.Ohm, M.Schmitt*, J.Bohl* and B.Lemmer*. Centers of Morphology and Pharmacology, J.W.Goethe Universität, 6000 Frankfurt, FRG.

A water soluble forskolin analogue [F]; forskolin-7-deacetyl-7-butyryl, Calbiochem] was used to activate adenylate cyclase [AC] in post mortem olfactory bulb, hippocampus and cerebellum of 7 controls (C) and 7 Alzheimer patients (ALZ). Diagnose were done by clinical and histopathological examinations. Basal as well as stimulated AC activity cAMP/ mg protein/min] was measured by formation of cAMP from 0.5 mM ATP in the presence of an ATP-regenerating system, suspensions incubated for 6 min at 37°C without and with 100 uM F, formed cAMP determined by radioassay. Protein was determined after Lowry. Data are means ± SEM. For statistics the non-parametric Mann-Whitney U-test was used.

	C	ALZ	Reduction	U-test
Olfactory Bulb				
Basal	57.6 ± 13.1	20.6 ± 3.3	64 %	0.002
100 uM F	209.7 ± 38.1	83.6 ± 17.9	60 %	0.003
Hippocampus				
Basal	27.0 ± 4.2	11.4 ± 2.5	58 %	0.001
100 nM F	192.9 ± 22.4	106.2 ± 13.4	46 %	0.002
Cerbellum				
Basal	27.8 ± 5.8	21.4 ± 3.5	23 %	n.s.
100 uM F	188.2 ± 33.4	137.9 13.4	27 %	n.s.

519.3

INTERLEUKIN-1 (IL-1), INTERLEUKIN-6 (IL-6) AND TUMOR NECROSIS FACTOR (TNF) IMMUNOREACTIVITIES ARE EXPRESSED IN MICROGLIA IN HUMAN BRAIN. D Dickson, L Mattiace, S-H Yen* and P Davies. Dept of Pathology (Neuropathology), Albert Einstein College of Medicine, Bronx, NY.

We have developed immunocytochemical methods for microglia in human brain to study the role of microglia in amyloidogenesis in aging and Alzheimer's disease. Microglia share a number of antigenic properties with macrophages, including presence of epitopes to class II major histocompatibility antigen (HLA-DR), Leu-M5 and leukocyte common antigen (LCA). Macrophages also produce a number of cytokines, including IL-1, IL-6 and TNF. In tissue fixed briefly in periodate-lysine-paraformaldehyde and sectioned with a Vibratome, microglia were immunoreactive with antibodies to IL-1, IL-6 and TNF. In Alzheimer's disease, the stained cells formed a reticular array in gray and white matter, and were clustered in areas of amyloid deposition. In control elderly brains, fewer microglia were immunostained. Double labeling studies showed that cytokine-reactive cells were microglia and not astrocytes, since they were co-labeled with antibodies to HLA-DR, Leu-M5 and LCA, but not antibodies to glial fibrillary acidic protein.

519.4

INTERLEUKIN-1ß mRNA LEVELS INCREASE IN ASSOCIATION CORTEX IN ALZHEIMER'S DISEASE. K. E. Rogers, A. B. Wadhams,* P. D. Coleman. Dept. of Neurobiology and Anatomy, University of Rochester, Rochester, N. Y. 14642 USA

Interleukin-1ß(IL-1ß) is a cytokine which has been shown to stimulate astrocyte proliferation. Additionally, it can induce expression of the ß-amyloid precursor gene. Thus, we have examined IL-1ß mRNA levels in superior frontal gyrus in normal aging and in Alzheimer's Disease(AD). Batch mRNA isolations were performed from 24 AD and nine age-matched control cases. Integrity of the mRNA was examined by Northern gel analysis and mRNA content was quantified by hybridization to an oligo dT probe. Serial dilutions of each sample were bound to a nylon membrane and probed with a cRNA transcript of human IL-1ß. Resulting autoradiograms were quantified by scanning densitometry. The total yield of mRNA per gram of tissue did not vary significantly between normal controls and AD cases. In control cases less than 80 years of age, Il-1ß transcripts were not detected. IL-1ß message was present in control cases 90 years and older, although at relatively low levels. In AD cases Il-1ß messages were present in all age groups. However, the IL-1ß message levels were higher in young AD cases than in AD cases 90 years and older. Thus, IL-1ß mRNA shows an age-related increase in normal aging and an age-related decrease in AD.(Supported by AG 09016, AG01121, AG 03644, AG 00107, PRG-89-120)

519.5

ANOMALOUS REACTION OF OLIGO PROBES FOR 3 HEAT SHOCK mRNAs IN ALZHEIMERS HIPPOCAMPUS. M. Morrison-Bogorad, S. Pardue*, K. Groshan*, C.L. White*, III, E.H. Bigio*, B. Border, E.K. Miller*, R. Gonzales* and J.D. Raese. Departments of Neurology, Biochemistry, Pathology and Psychiatry, University of Texas Southwestern Medical Center, Dallas, TX, 75235, and The Schizoprenia Research Center, Veterans Administration Medical Center, Dallas, TX, 75216.

The stress protein, ubiquitin, is expressed in some affected neurons in Alzheimers disease (AD). Little is known about the expression of other members of the heat shock family in AD. We hybridized oligo probes specific for human hsx70, hsp70 and hsc70 mRNAs to cerebellum and hippocampus from 3 control and 7 AD brains. Sections were counterstained with congo red to demonstrate AD pathology. All 3 probes showed a strong signal, highest for the hsc70 probe, in CA1-2, subiculum and entorhinal cortex of 6/7 AD hippocampi. These regions contained plaques and tangles. Grains were present in pyramidal neurons and were often associated with neurofibrillary tangles. No comparable reaction was present in either region of control brain or in AD cerebellum. Post-hybridization washing at high stringency (0.5 XSSC, 45°C) reduced but did not abolish signal. In 6/7 cases, signal intensity was markedly increased when sections were pretreated with ribonuclease. In contrast, a control 18S rRNA oligo showed normal cell-specific hybridization to each brain that was always completely eliminated by ribonuclease pretreatment. Further experiments will determine whether the anomalous reaction with the heat shock probes in affected regions of AD hippocampus is true hybridization and whether it has any mRNA-specificity. Supported by NIH grant AG08013 and by the Leland Fikes Foundation.

519.6

A DEVELOPMENTALLY EXPRESSED PROTEIN ISOLATED FROM NEONATAL RAT NEOCORTEX. M. Murtaugh, R. Maroko, J. Rabii, and M.W. Miller. Dept. of Anatomy, Univ. of Med. and Dent. N.J.-Sch. of Osteopathic Med. and R.W. Johnson Med. Sch., Piscataway NJ 08854, and Prog. in Physiol. and Neurobiol., Rutgers Univ., Piscataway NJ 08854.

Alz-50 is a monoclonal antibody which recognizes an antigen expressed in the cerebral cortices of patients with Alzheimer's Disease and Down's Syndrome and of normal human fetuses. An Alz-50-positive antigen is virtually absent from the cortices of normal human adults. A recent immunohistochemical study has shown that Alz-50 also identifies a population of cortical neurons in neonatal rats. We isolated the antigen recognized by Alz-50 in rat cortex using immunoaffinity column chromatography; the column was bound with Alz-50 and eluted with 3.0 M potassium isothio-cyanate. Unlike the Alzheimer's antigen (M.W. 68 kDa), the isolated rat protein had a molecular weight of about 50 kDa. This antigen was evident during the first postnatal week and its expression waned during the second postnatal week. Both the molecular weight of the neonatal antigen and the timing of its expression are reminiscent of rat juvenile tau. Funded by DE 07734 and AA 06916.

519.7

ANALYSIS OF GENE EXPRESSION IN ALZHEIMER'S DISEASE USING THE POLYMERASE CHAIN REACTION. T. Golde, M. Cohen* T. Cheung, S. Estus, C. Hopfer*, R. Kalaria, L. Younkin, and S. Younkin. Case Western Reserve University, Cleveland, OH 44106

To analyze the role of altered gene expression in the evolution of AD pathology, it is essential that methods be developed to analyze multiple mRNAs in the samples that can be obtained from discrete brain regions wherein pathology is well defined. In a previous study, we analyzed alternatively spliced ß amyloid protein precursor (ßAPP) mRNAS quantitatively by using the polymerase chain reaction to amplify ßAPP cDNAs produced by reverse transcription. Since this method appears to be well suited for the analysis of gene expression in AD, we have synthesized oligonucleotide primer pairs specific for ßAPP, ß-actin, cyclophillin, GFAP, alpha-1-antichymotrypsin, serum amyloid P, IL1-ß, MARCKS, and c-raf mRNAs. Using these primer pairs, we have amplified cDNAs specific for each mRNA from 1 µg or less of randomly primed reverse transcribed total RNA from AD or control brain, and we have confirmed by direct sequencing that these cDNAs do, in fact, correspond to the mRNAs targeted. Optimization of the amplification process has enabled us to simultaneously amplify up to 6 different cDNAs from 1 µg of RNA with minimal amplification of non-specific products. Moreover, by diluting randomly primed white matter cDNA, we find that the relative amounts of up to 5 different messages can be quantitated in a single PCR reaction as long as amplification is in the exponential phase and the messages are roughly equal in abundance. Furthermore, by comparing the relative amounts of 87 bp and 300 bp ß-actin cDNAs produced with appropriate primers, it is possible to evaluate the extent of degradation present in each postmortem RNA sample and to select, on the basis of the 87 bp / 300 bp ratio, samples in which degradation has minimal effect on the level of the mRNAs measured. We are currently using this method to analyze expression of many mRNAs in various regions of AD and control brains.

519.8

EPIDERMAL GROWTH FACTOR RECEPTOR EXPRESSION IN BRAIN, PITUITARY AND SKIN OF DEMENTED AND NONDEMENTED ELDERLY PATIENTS. S. D. Styren, E. J. Mufson, W. H. Civin and J. Rogers. L. J. Roberts Center, Institute for Biogerontology Research, Sun City AZ. 85351

Epidermal growth factor receptor (EGFR) is a 170KD integral membrane protein which contains a tyrosine kinase moiety capable of phosphorylating a variety of cellular proteins related to cellular proliferation. Stimulation of the tyrosine kinase can be accomplished by the binding of Vaccinia growth factor and transforming growth factor in addition to epidermal growth factor. We have previously reported the presence of EGFR on brain vascular endothelial cells in patients presenting with a variety of dementias, and the absence of EGFR immunoreactivity in neurologically normal or nondemented Parkinson's patients. In the present study we evaluated the distribution of pituitary (n=9), scalp (n=11), and abdominal skin (n=11) EGFR in nondemented (neurologically normal and Parkinson's disease) and demented patients (Alzheimer's, Parkinson's, multi-infarct, and Pick's dementias).

Vascular EGFR immunoreactivity in the pituitary is strongly correlated with dementia. Three of three demented patients exhibited strong pituitary vascular EGFR immunoreactivity, whereas 5 of 6 controls lacked EGFR expression. The one anomalous control patient had moderately high neuritic plaque and tangle pathology. EGFR immunoreactivity in scalp and abdominal skin is less consistently correlated with dementia than brain or pituitary EGFR. EGFR positive blood vessels were present in scalp in 8 of 12 demented patients, and in abdominal skin in 4 of 6 demented patients. Scalp (n=6) and abdominal skin (n=4) from nondemented controls lacked vascular EGFR immunoreactivity. These findings indicate that in demented patients the CNS may be a source of an EGFR inducing substance, or that vascular endothelial cells may have differential sensitivity to the inducing substance depending on their systemic location.

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519.9

ISOFORMS OF RIBOSOMAL PROTEINS ASSOCIATED WITH ALZHEIMER'S DISEASE. J.Gotlib*, D.Vanderputten*, N.Khowong*, R. Poglod*, V. Haroutunian, L.Bierer, D.Perl, C.Merril, and W. Wallace Dept.Psychiatry and Center for Neurobiology, Mt. Sinai Med. School, New York and Lab. of Biochem. Genetics, NIMH, Washington, D.C.

We have shown previously that polysomes isolated from AD tissues exhibit reduced in vitro synthesis of proteins. Ribosomes were purified from control and AD polysomes and characterized to determine the mechanism responsible for this disruption. Control and AD ribosomes equally support translation of hemoglobin mRNA in the in vitro translation assay (69.2 ±17 vs. 76.4 ±44 X 10^6dpm 35S-met|A260 unit, n= 5). Likewise, the physical integrity of ribosomal RNA appeared similar by northern analysis of 18S rRNA. However, examination of ribosomal proteins by two dimension gels indicate three polypeptides that are consistently different in the AD ribosomes (50 kD,pI 7.8; 30 kD,pI 7.8; and 16 kD pI 8.2). These differences appear to be due to alterations in the predominance of isoforms of ribosomal proteins. Such alterations may represent differences in post-translational modifications of the proteins, such as protein phosphorylation. The altered proteins are currently being identified and their phosphate content, if any, measured.

519.10

NUCLEAR RUN-ON AS MEASURED IN NUCLEI FROM FROZEN BRAIN TISSUES: AN APPROACH TO MEASURING TRANSCRIPTION IN HUMAN BRAIN REGIONS. S.A. Johnson, S. Millar* and C.E. Finch. Andrus Gerontology Center and Dept. of Biol. Sci., Univ. So. Cal., Los Angeles, CA 90089.

Nuclear run-on analyses give in vitro measurements of relative transcription rates for individual genes. As shown in numerous other tissues, nuclear run-on can be used to monitor rapid transcriptional changes in response to various experimental stimuli. To study the effects of Alzheimer's disease on specific gene transcription, we have developed nuclear run-on transcription assays using nuclei isolated from**frozen** brain. Initial studies showed no difference in yield of nuclei per tissue mass, or amount of run-on transcript produced, between nuclei from fresh or frozen rat brain. Transcription was dependent upon added ribonucleotide triphosphates. Freezer storage time had no apparent effect upon the yield of nuclei or labeled run-on transcript produced. Nuclei isolated from frozen human post-mortem brain produced equivalent amounts of run-on transcript per isolated nucleus, as compared to fresh or frozen rat brain nuclei. Brain cell nuclei can be separated by sucrose gradient centrifugation into discrete fractions that containlarge vs small nuclei; large nuclei are thought to be of neuronal origin, while the fraction containing small nuclei represents glia and small neurons (Sarkander and Uthoff, FEBS Lett., 15, 53, 1976). Preliminary experiments show hybridization of run-on transcript to cDNAs for b-tubulin (neuronal and glia) and tyrosine hydroxylase (neuronal), but not for GFAP (astrocyte). The small nuclei transcript showed hybridization to GFAP cDNA. This now allows discrimination of neuronal and glial transcription rates in human brain. Supported by NIA AG07909 to C.E. Finch.

519.11

THE PROTEIN ENCODED BY pADHC9/SGP2 IS EXPRESSED IN ALZHEIMER'S DISEASE HIPPOCAMPUS AND IS ELEVATED IN THE RAT HIPPOCAMPUS FOLLOWING ENTORHINAL CORTEX LESION

M. Lampert-Etchells, P.C. May, N.J. Laping, S.K. Schrieber, C.E. Finch. Andrus Gerontology Center, University of Southern California, Los Angeles, CA 90089

Clone pADHC-9 encodes a 2 Kb RNA which is overexpressed in Alzheimer's disease (AD) hippocampus and is the human counterpart to rat testicular SGP2 (Jenne, D.E., Tschopp, J.,(1989) P.N.A.S. 86, 7123) Polyclonal antiserum was made to the human pADHC9/SGP2 protein to study the level of expression, the cellular distribution, and the function of this protein in Alzheimer and normal hippocampus. An XhoII fragment of pADHC9/SGP2 from nucleotide 921-1500 was inserted into the E. coli expression vector pATH-1. The fusion protein made from this vector was used to generate rabbit antibodies to pADHC9/SGP2 protein. Western blot analysis with the IgG fraction identified the expected 40kD reduced protein in human serum and identified a similar 40kD protein in human spinal fluid. In human Alzheimer and control hippocampus extracts this antiserum identified the 38kD reduced form of this protein along with its 55kD precursor. The pADHC9/SGP2 protein bands migrated as typical diffuse glycoprotein bands.

In the rat, entorhinal cortex lesion was used as a model for aspects of the hippocampal degeneration seen in AD. By northern blot analysis SGP2 mRNA was elevated in the hippocampus several fold by 2 days post lesion. It reached a peak at 6 days and decreased to normal levels by 14 days post lesion. The SGP2 protein at 4 days post lesion was also increased relative to the unlesioned contralateral hippocampus. We conclude that SGP2 mRNA and protein respond dynamically to brain lesions and give a new probe for molecular changes in AD. Supported by ADRDA IIRG-88-069 and NIH/NIA AG07909.

FRIDAY AM SYMPOSIA

521

SYMPOSIUM: GLIA: SYNTHESIS OF AND RESPONSES TO GROWTH FACTORS AND NEUROPEPTIDES. J.P.Schwartz, NINDS, NIH (Chairperson); D.E.Brenneman, NICHD, NIH; M.Dubois-Dalcq, NINDS, NIH; F.A.McMorris, Wistar Inst.; W.S.T.Griffin, Univ.Ark.Med.Sci.

Communication between neurons and glia can affect differentiation, mitosis and other processes: evidence will be presented that such communication can be mediated by both growth factors and neuropeptides. **D.E.Brenneman** will show that vasoactive intestinal peptide, a neuropeptide released from neurons, acts as a secretagogue for neurotrophic factors from glia. **J.P.Schwartz** will demonstrate developmental expression and regional specificity for neuropeptide synthesis by astrocytes. **M.Dubois-Dalcq** will compare effects of two growth factors, platelet-derived growth factor and basic fibroblast growth factor, on O-2A oligodendrocyte progenitor cells. **F.A.McMorris** will show that the insulin-like growth factors regulate three key steps in myelination by oligodendrocytes, with data from cell and organ cultures as well as transgenic mice. **W.S.T.Griffin** will show that the levels of interleukin-1, a microglia-derived astrocyte mitogen, and S100ß, an astrocyte-derived neurotrophic factor, are elevated in Alzheimer and Down syndrome brain.

522

SYMPOSIUM: PHYSIOLOGY OF PEPTIDERGIC NERVE TERMINALS IN THE VERTEBRATE NEUROHYPOPHYSIS.

C.W. Bourque *McGill University (chairperson)*, J.T. Russell *N.I.H.*, A.L. Obaid *U. Pennsylvania*, B.M. Salzberg *U. Pennsylvania*, M.C. Nowycky *Med. Col. of Pennsylvania*, J.R. Lemos *Worcester Fndn. Exptl. Biol.*

The hypothalamo-neurohypophysial system of vertebrates consists of the hypothalamic somata of magnocellular neurons synthesizing either oxytocin or vasopressin and their axonal projection to the posterior pituitary. Because of their relatively large size (several µm) and compartmentalization, neurohypophysial terminals have become a classic preparation to examine the physiology of peptide secreting terminals. This symposium will highlight a number of convergent advances in understanding the mechanisms that modulate the coupling between axonal firing, nerve terminal excitation and secretion from this neuroendocrine system. Russell will review the effects of activity patterns on neuropeptide secretion from the neurohypophysis, and describe the modulation of intraterminal [Ca^{2+}] signals in isolated terminals. Bourque will relate the activity-dependence of spike invasion and excitation of nerve terminals to the effects of firing patterns on peptide secretion. The mechanisms of Ca^{2+} influx in the intact terminals of the neurohypophysis as probed with potentiometric dyes will be described by Obaid. Salzberg will describe changes in light scattering consequent to nerve terminal excitation and detail their relation to excitation-secretion coupling. Nowycky will describe patch clamp studies of neurohypophysial Ca channels in isolated terminals and discuss their role in exocytosis as studied with capacitance measurement techniques. Finally, Lemos will characterize ion channels in isolated terminals and neurosecretory granules, and comment on their possible role in the control of peptide secretion.

SENSORY SYSTEMS—VISUAL CORTEX: INTRACORTICAL INTERACTIONS

523.1

INTER-AREAL AND INTER-HEMISPHERIC SYNCHRONIZATION OF OSCILLATORY RESPONSES IN CAT VISUAL CORTEX.

A.K. Engel*, P. König*, A.K. Kreiter* and W. Singer. Max-Planck-Institut für Hirnforschung, Deutschordenstr.46, 6000 Frankfurt 71, F.R.G.

We have previously demonstrated that oscillatory responses can synchronize across orientation columns in area 17 of cat visual cortex. We proposed that this synchronization may provide a temporal coding mechanism for scene segmentation. This hypothesis predicts that 1. similar oscillations should occur in other visual areas, 2. there should be evidence for inter-areal synchronization and 3. inter-hemispheric synchronization should be observed. To test these predictions, we made simultaneous recordings of multi-unit activity from area 17 and PMLS in 4 adult cats. In 3 additional cats, we recorded simultaneously from area 17 of either hemisphere. The oscillatory nature of the responses and the strength of synchronization were quantified by computation of auto- and cross-correlograms. We obtained the following results: 1. More than 70% of the PMLS recordings displayed an oscillatory modulation in the frequency range of 40-60Hz. 2. In more than half of the cases tested, we observed a response synchronization between area 17 and PMLS. 3. The inter-areal response synchronization depends on the overlap of the receptive fields, but not on differences in orientation preferences. 4. The 17-PMLS interaction is sensitive to global stimulus features, such as continuity of contours and coherence of motion. 5. In 70% of the cases where we recorded simultaneously from area 17 of both hemispheres, we observed a response synchronization. The interactions are in strength comparable to those within area 17. 6. We observed phase-locking also between cells with non-overlapping fields and dissimilar orientation preferences. Our results support the hypothesis that oscillations in the range of 40-60Hz may be a general phenomenon in cat visual cortex and that response synchronization can involve cells located in different hemispheres or visual areas. The inter-areal synchronization may serve for the binding of different feature maps. The inter-hemispheric interactions may provide a mechanism to link features of objects extending across the vertical meridian.

523.2

SQUINT AFFECTS OCCURRENCE AND SYNCHRONIZATION OF OSCILLATORY RESPONSES IN CAT VISUAL CORTEX.

Peter König*, Andreas K. Engel*, Siegrid Löwel* and Wolf Singer. Max-Plank-Institut für Hirnforschung, Deutschordenstr. 46, 6000 Frankfurt 71, F. R. G.

We could recently demonstrate that neurons in cat visual cortex display responses with an oscillatory temporal structure in the range of 40-60Hz which can synchronize over distances of 7mm in area 17. This synchronization may serve to establish relations between features in different parts of the visual field. We have now investigated occurence and synchronization of oscillatory responses in area 17 of strabismic cats. We wondered whether in these animals response synchronization primarily occurs between cells of similar ocular dominance (OD).

We recorded multi-unit activity (MUA) to appropriately oriented moving light bars simultaneously from 4-6 spatially separate sites in cortical area 17. We used 4 adult cats in which divergent strabismus had been surgically induced at the age of 3 weeks. To determine the temporal relationship of the firing patterns, we computed auto- and crosscorrelation functions of the spike trains.

Analysis of 112 recording sites gave the following results: 1) 87% of the responses were monocular or strongly dominated by one eye. 2) The occurrence of oscillatory responses was with 63% as frequent as in normal cats, but response rhythmicity was much weaker. 3) Only 30% of the 115 pairs of MUA recordings showed a response synchronization versus 59% for overlapping receptive fields in normal cats. 4) Response synchronization occurred primarily between cells with the same OD and less frequently between cells with different OD.

Our data support the hypothesis that the functional organization of area 17 is profoundly altered in strabismic cats. The weak oscillatory activity might reflect disturbed integrative capacities in the visual cortex of squinting animals. The preferential synchronization between neurons driven by the same eye suggests that cells with different OD become functionally independent, which implies not only changes in thalamo-cortical but also in cortico-cortical connectivity.

523.3

Temporal Dynamics of Oscillatory Neuronal Interactions in Cat Visual Cortex. Charles M. Gray, Andreas K. Engel*, Peter Koenig* and Wolf Singer, Max-Planck-Institute for Brain Research, 6000 Frankfurt/M 71, F.R.G.

Previously we have demonstrated that a subpopulation of visual cortical neurons exhibit oscillatory responses to their preferred stimuli at a frequency near 50 Hz (Gray and Singer,PNAS,1989). These responses can selectively synchronize over widespread areas of cortex in a stimulus-specific manner (Gray,et al.,Nature,1989). Here we report the results of a new analysis which reveals the fine temporal structure inherent in these interactions. We utilized pairs of recordings of the local field potential (LFP) activity from area 17 in the cat which met two criteria: The LFP was correlated with the underlying unit activity at each site and the recording sites were at least 5 mm apart in cortex. A moving window technique was applied to compute time-lagged cross-correlograms on 100 msec epochs of data repeated at intervals of 30 msec for a period of 3 sec during each direction of stimulus movement. And a statistical test was devised to determine the significance of detected correlations. In this way we were able to determine the magnitude, phase-lag, frequency and duration of correlation events as well as an estimate of the time needed to achieve phase-locking. The results demonstrate that 1) synchrony can be established within 1-2 cycles of oscillation, 2) the duration of synchrony is variable and lasts from 50-500 msec, 3) the phase and frequency of synchronized events is also variable within and between events and ranges from +- 3 msec and 40-60 Hz, respectively, 4) multiple correlation events can occur within a single stimulus period. These results demonstrate a high degree of dynamic variability among interacting populations of neurons which is consistent with the requirements of a mechanism for feature integration.

523.4

ORIGINS OF OSCILLATORY ACTIVITY IN THE CAT'S VISUAL CORTEX. G.M. Ghose*, R.D. Freeman. Biophysics and Neurobiology Groups, Univ. of Calif., Berkeley, CA 94720.

The activity of neurons in the visual cortex, generally studied by spike activity or gross potentials, has been reported to exhibit oscillatory firing patterns. We have analyzed spike trains of cortical neurons and lateral geniculate fibers in the visual cortex of the cat under different types of visual stimulation in order to ascertain the nature and origin of these oscillations.

Two types of visual stimuli were presented for extended periods of time to determine the stability and stimulus dependence of oscillatory firing. The first type consists of large drifting gratings of sinusoidal intensity at optimal orientations and spatial frequencies. The second type of stimuli consists of random sequences of small optimally oriented bright and dark bars flashed for 50 ms. at different locations within and around the receptive fields. For both stimuli power spectra of extracellularly recorded action potentials are computed to provide quantitative assessments of the degree of oscillatory firing.

Oscillatory firing, as exhibited by a peak above noise at around 50 Hz in the power spectrum, is observed in 35% of the cortical cells and LGN fibers studied by grating stimulation. The oscillations of several LGN fibers are among the strongest found. Oscillations are generally highly variable over a period of seconds in both frequency and magnitude. Moreover, the relative magnitude of this oscillatory firing is sometimes inversely related to firing rate. These findings suggest that such oscillations are at least in part due to intrinsic spontaneous firing properties. Oscillations do not depend on the presence of large coherent stimuli, since 30% of the cells studied with small bars also fire rhythmically at around 50 Hz. In conjunction with previous studies in the LGN and retina, these results support the notion of a subcortical, rather than intracortical, origin of oscillatory firing. (EY01175)

523.5

MODULATION OF CLASSICAL RECEPTIVE FIELD RESPONSES BY MOVING TEXTURE BACKGROUNDS IN MONKEY STRIATE CORTEX: SPATIAL AND TEMPORAL INTERACTIONS. J.M. Fox, T. Delbruck*, J.L. Gallant, C.H. Anderson*, and D.C. Van Essen. Div. of Biology 216-76, Caltech, Pasadena, CA 91125.

We have examined the effect of texture patterns moving outside the classical receptive field (CRF) on the spatial sensitivity profile of single units in striate cortex (V1) of anesthetized macaque monkeys. Our stimuli consisted of a target and a moving background. The target was an optimally oriented line segment flashed at different locations along an axis orthogonal to the preferred orientation within the CRF, and in different phases relative to the background motion. The background was a random dot pattern lying entirely outside the CRF and oscillating at a temporal frequency of 2Hz along this axis. Responses were compared for 3 to 5 positions of the flashed target, and across 4 phases of the background motion. We also examined responses to targets flashed in the absence of a background, and to moving backgrounds alone.

In the 35 cells subjected to detailed analysis thus far, we found that approximately half (17/35) showed a significant modulation of the responses to the target by the moving background. The background caused enhanced responses to the target in some instances and suppression in others; the magnitude of the effect was two-fold or more in some cells. In the majority of cells the background produced enhancement or suppression only within a subset of the spatial locations and phase conditions tested. In a minority of cells there was a differential effect, such that targets presented at a particular phase were enhanced at one target location and suppressed at another. These observations suggest that complex spatio-temporal interactions between target and background occur in V1.

523.6

SPATIAL ORGANIZATION OF SUPPRESSIVE SURROUND EFFECTS IN NEURONS OF AREA V1 IN ALERT MACAQUES. J. J. Knierim and D. C. Van Essen. Caltech, Division of Biology 216-76, Pasadena, CA 91125.

In a majority of neurons in V1, responses to a line segment within the classical receptive field (CRF) are suppressed by a texture pattern lying entirely outside the CRF. We studied the spatial organization of these surround effects by presenting texture surrounds to the quadrants along the flanks of the CRF and separately to the quadrants at the ends of the CRF. The orientation of the line segments in the surround was made either identical to that in the CRF (uniform orientation) or orthogonal to it (orientation contrast), since we have previously shown that orientation contrast reduces the suppressive effect in many cells (Knierim & Van Essen, *Soc. Neurosci. Abstr.*, 1989).

For the sample of 122 cells tested, both the flank quadrants and the end quadrants suppressed the response by an average of 25%, which was smaller than the 35% suppression induced by the full surround. Thus, the surround effects are spatially distributed and not exclusively attributable to end-stopping or to side-band suppression. In each case, the suppression induced by the uniform orientation surround was about 9% greater than that induced by the orientation contrast surround. However, we did encounter a spatial asymmetry for the subset of 39 cells that showed a significantly greater response to orientation contrast than to uniform orientation on the full-field tests. In these cells, for the end quadrants, the uniform orientation surround suppressed the response 17% more than the orientation contrast surround, whereas for the flank quadrants, the difference in suppression was 9%. Thus, the orientation contrast effect originates from both the end zones and the flanks, but more strongly from the former.

We also recorded responses to a full-field texture in which the surrounding line segments were randomly oriented. This stimulus is similar to the uniform orientation surround in that there is no orientation contrast between the line segment in the CRF and those outside. Of the 50 cells tested, 9 responded more strongly to the orientation contrast texture than to the random orientation texture, whereas only 3 responded in the opposite fashion. These differences were comparable to those for the uniform orientation comparison. Thus, the differential effects depend on the overall presence or lack of orientation contrast, rather than on the presence of a uniform orientation surround.

523.7

SINGLE UNIT AND 2-DEOXYGLUCOSE STUDIES OF MULTI-CYCLE INHIBITION IN MACAQUE STRIATE CORTEX. R.T. Born and R.B.H. Tootell, Dept. of Neurobiol., Harvard Medical School, Boston, MA.

In the course of single unit studies to map spatial frequency tuning in supragranular striate cortex of the macaque monkey, we discovered that a large population of neurons in the interblob regions responded poorly or not at all to extended gratings, but gave vigorous responses to single bars or edges. To characterize this property we have recorded from 93 single units in the interblobs of layers 2 and 3 in striate cortex of the anesthetized, paralyzed macaque.

In these cells, gratings became effective stimuli only when they were spatially delimited from the sides so that the grating had fewer cycles. This property, which we call multi-cycle inhibition, was present in 65 (70%) of the neurons we studied. It was independent of and more common than end-stopping (27%). Thirty-three cells studied quantitatively gave a maximal response to 1.3 +/- 0.6 (mean +/- sd) grating cycles and a half-maximal response to 3.5 +/- 1.7 grating cycles. In a few cells tested with separate center and surround grating patches, the inhibition occured only when the orientation of the surround grating was similar to that of the center.

In 2 animals, 2-deoxyglucose was used to compare brain activation produced by a sine-wave grating vs. a rectangular grating (same white bar width but 60% fewer bars), in retinotopically discrete regions. As one would expect from the single unit evidence, interblob cells in layers 2 and 3 of striate cortex had higher uptake when stimulated by the rectangular grating. In other layers, uptake produced by the two stimuli was equal (5 and 6) or greater for the sine-wave grating (4C). Subsequent single unit studies have shown that the 2-deoxyglucose result in not due to low-pass modulation transfer functions of the interblob cells.

Multi-cycle inhibition is a short-range spatial antagonism between contrasts of like orientation. We think of these cells as "contour-pass filters" that may help to discriminate highly redundant, texture-like regions of a visual scene from contours representing object boundaries. Supported by grants NIMH 14275 and EY 07980.

523.8

GABA-INDUCED LOCAL INACTIVATION AND CROSS-ORIENTATION INHIBITION IN AREA 18 OF THE FELINE VISUAL CORTEX.
J.M. Crook and U.T. Eysel. Department of Neurophysiology, Faculty of Medicine, Ruhr-Universität Bochum, D-4630 Bochum, F.R.G.

We investigated the influence of iontophoresis of GABA through four pipettes, each located at a horizontal distance of some 500–600μm from the recording site, on the orientation tuning of 74 cells in area 18 of cat visual cortex. Sixty-one percent showed significant broadening of orientation tuning during GABA iontophoresis, with a 79% increase in mean tuning width. The results were quantitatively similar to those from comparable experiments in area 17 (Crook et al., *Eur. J. Neurosci.* 2:259, 1989; Eysel et al., *Soc. Neurosci. Abstr.* 15:324, 1989). Since the tangential distance between recording and inactivation sites was approximately half that required for a complete sequence of preferred orientations, we interpret the results in terms of a loss of 'cross-orientation' inhibition during GABA iontophoresis.

To provide more direct evidence on this point, we have more recently determined the orientation and directional specificity, ocular dominance and receptive field limits of single and multi-unit activity at the inactivation sites, before applying GABA. Depending on the orientation preference at each inactivation site, GABA was applied either simultaneously through all four pipettes or selectively through one or a number of pipettes. The initial results from these experiments support our original conclusion that inhibition between cells with overlapping receptive fields and dissimilar preferred orientations enhances the orientation selectivity of cells in cat visual cortex.

523.9

RELATIONSHIP BETWEEN EXCITATORY AND INHIBITORY LONG RANGE CONNECTIONS IN THE CAT VISUAL CORTEX. Z.F. Kisvárday and U.T. Eysel. Dept. Neurophysiology, Ruhr-University Bochum, Pf. 102148, D-4630 Bochum 1, FRG.

To study long range intracortical connections the neuronal tracer biocytin was focally injected into areas 17 or 18 after recordings at several sites. The labelling was analyzed in horizontal sections. Labelled cells and axons with Golgi-like appearance were found in distinct patches 0.8-3.5mm from the injection site. Axons and dendrites of identified cells were reconstructed, and their distribution compared between labelled patches.

Most frequently, pyramidal and spiny stellate cells, but occasionally smooth dendritic cells of the large basket cell types were encountered in layers 2-5. Axons of long range pyramidal and spiny stellate cells terminated predominantly in 3-6 distinct patches providing similar feed-back connections to the same and other labelled patches. In contrast, basket cells with comparable axonal extent did not form patches, but instead arborized in large horizontal slabs terminating primarily outside the area covered by the axons of other labelled spiny cells.

We conclude that some of the long range reciprocal connections allow integration of information over a greater area of the visual field than that covered by their classical receptive field. GABAergic large basket cells could enhance the specificity of these interactions by inhibiting populations of cells outside these patches.

523.10

EXTRINSIC AND INTRINSIC INHIBITORY CONNECTIONS IN RAT VISUAL CORTEX. C.T.McDonald and A.Burkhalter. Dept. of Neurosurgery and McDonnell Center for Higher Brain Function, Washington Univ. Sch. of Med. St.Louis, MO 63110.

Intrinsic long-distance interactions and feedback inputs from higher cortical areas are two mechanisms proposed to account for some of the receptive field properties seen in primary visual cortex (area 17). We have examined whether inhibitory neurons participate in such circuits by combining retrograde labeling and immunocytochemistry with a monoclonal antibody to glutamic acid decarboxylase (Chang and Gottlieb, J.Neurosci. 8:2123, 1988).

Following injection of fluorescent latex microspheres into area 17, double labeled GABAergic neurons can be identified in flat mount sections several millimeters lateral to the injection site in the extrastriate visual area 18a. In parasagittal sections through area 17, local inhibitory neurons are seen in layers 1-6 within 0.3mm of the injection tract. However, a second population of double labeled cells with much more extensive collateral arbors are seen at the layer 5/6 border up to 1mm from the injection site. Occasionally, cells with a simlar distribution are also encountered in layer 2/3.

These results suggest that neurons in primary visual cortex are under direct inhibitory feedback control from specific higher visual areas. They also suggest that direct, inhibitory, long-range connections do exist in rat primary visual cortex and that they are mediated by neurons with a unique laminar distribution.

Supported by grant NIH EY05935.

523.11

ORGANIZATION OF EXCITATORY LOCAL AXON COLLATERALS WITHIN RAT VISUAL CORTEX. R.R.Johnson and A.Burkhalter. Dept. of Neurosurgery and McDonnell Center for Higher Brain Function, Washington Univ. Sch. of Med., St. Louis, MO 63110.

Amino acid mediated excitatory neurotransmission plays an important role in the plasticity of intracortical circuits in developing and adult animals. To identify the cells which participate in these processes and to study the organization of their axon collaterals we have used retrograde tracing with D-^{3}H-aspartate in rat primary visual cortex.

Injections into superficial and middle layers labeled cells in layers 2-6 in a stereotypical horizontal and sublaminar distribution pattern. The projections within layer 3 and from the top and the bottom of layer 5 were much wider (0.75-1.5mm) than those from layers 4 and 6 which were contained within a ~0.5mm wide column. Typically, labeling in layer 2 and the corticotectal output zone in the middle of layer 5 was sparse. Alternatively, injections into deep layers produced a different labeling pattern and revealed long-range connections (~1mm) within the bottom of layer 5 and the top of layer 6. The projections from layers 2-4 and the corticogeniculate output zone in the lower half of layer 6 were narrow (<0.5mm) and topographically precise. Interestingly, cells at the grey/white matter border in layer 6 which are known to project widely throughout all layers were always confined to a small region below the injection site. These results suggest that we have selectively labeled glutamatergic/aspartergic neurons and that they participate in specific circuits which mediate short and long-range interactions.

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523.12

INTERACTIONS AND STIMULUS-DEPENDENT CHANGES OF SYNAPTIC POTENTIALS EVOKED BY ACTIVATING INTERLAMINAR AND HORIZONTAL PATHWAYS IN THE CAT'S STRIATE CORTEX.

J.A. Hirsch and C.D. Gilbert. Laboratory of Neurobiology, The Rockefeller University, New York, NY 10021.

Interlaminar and long-range horizontal pathways converge on single cells in the cat's primary visual cortex. To gauge the effects and interactions of these two sources of input on their target neurons, we made intracellular recordings from layer 2+3 in slices of area 17. Pyramidal cells, identified by injection of biocytin, responded to electrical shocks delivered both to layer 4, in vertical alignment with the recording electrode, and to those applied at a horizontal distance of $\geq$1 mm in layer 2+3. Responses from each site appeared to arise largely through separate circuits since fatigue of one by tetanization rarely influenced the other. Moreover, simultaneous activation of interlaminar and horizontal paths produced summation.

Long-lasting changes in synaptic responses could be produced by repetitively pairing EPSPs activated from one or the other site with the injection of suprathreshold pulses of depolarizing current. Following conditioning with 10 to 50 pairs presented at frequencies of 2-5 Hz, facilitation of the paired response was observed for up to 2.5 hours. For some cells, after facilitating one of the two paths, the second could be conditioned and strengthened as well. The enhancement was most pronounced in polysynaptic EPSPs and was greatly reduced by blocking NMDA receptors. We are currently exploring whether facilitation of one pathway can alter the efficacy of the other. (Supported by NIH grant NS22789 and NSF grant BNS8918951)

CELL LINEAGE III

524.1

SPECIFICATION OF RETINAL CELL PHENOTYPES: EVIDENCE FOR A DEFAULT PATHWAY OF PHOTORECEPTOR DIFFERENTIATION. **A. Repka*, and R. Adler. Wilmer Institute, Johns Hopkins Univ. Sch. of Med., Baltimore, MD. 21205.**

Previous studies led us to hypothesize that retinal precursor cells remain plastic after terminal mitosis. Unless induced to develop as neurons by intraretinal signals, cells will follow a photoreceptor "default pathway" (*Science*, 243:391-393, 1989). This hypothesis predicts that precursor cells that undergo terminal mitosis *in vitro*, and therefore differentiate in the absence of intraretinal signals, should give rise predominantly to photoreceptors. We tested this prediction by *in vitro* labeling of dividing precursor cells dissociated from embryonic day (ED)5-8 chick retinas, using ^{3}H-thymidine or BUDR. Their fate was determined by phase contrast microscopy, opsin immunocytochemistry and sequential photography. Labeling indices were highest during the first day *in vitro*, with cells becoming postmitotic on *in vitro* days 2-3. In all cases, 80-95% of the differentiated cells "born" *in vitro* showed a photoreceptor phenotype, regardless of the phenotypes of neighboring cells that were already postmitotic at the time of their isolation for culture. These observations are consistent with and add support to the photoreceptor default pathway hypothesis.

524.2

A RETINAL ANTIGEN WITH ALTERED STAINING PATTERN DURING METAMORPHOSIS IS ALSO EXPRESSED BY NEURAL CREST. S. Hoskins and G. Kirchbaumer.* Department of Biology, City College of New York, NY, NY 10031.

Using immunosupression methods, we generated IPS6, an antibody whose expression appears to be developmentally modulated during both metamorphosis and embryogenesis in *X. laevis*. In premetamorphic retinas, IPS6 stains pigment epithelium, photoreceptor outer segments throughout the retina, and the peripheral germinal zone, which contains retinal stem cells. During metamorphosis, staining is lost from central retina, becoming restricted to the pigment epithelium and the peripheral stem cells. This change in staining pattern correlates temporally with metamorphosis, but appears to be independent of thyroid hormone. Blocking metamorphosis does not prevent the loss of immunoreactivity in central retina, nor does intraocular injection of thyroxine accelerate the change in staining pattern.

Although IPS6 was raised against retina from young postmetamorphic frogs, it also stains the eye rudiment, ectoderm overlying the eye, and neural crest of embryos. Both neural crest and the retinal germinal zone are stem cell populations, and in each, individual precursors may give rise to pigment cells as well as to neurons. Work in progress is aimed at determining why immunoreactivity is lost from central retina, and the significance of IPS6 co-expression by precursor cells of both the central and peripheral nervous system.

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524.3

NEURONAL LINEAGE AND DETERMINATION IN THE CHICK RETINA USING RETROVIRUSES AND CELL TRANSPLANTS. D.M. Fekete, E.F. Ryder, A.W. Stoker*# and C.L. Cepko. Harvard Medical School, Boston, MA 02115 and #Lawrence Berkeley Lab., Berkeley, CA 94720.

Clonal analyses in the retinas of rodents and frogs have shown that different cell types can arise from a common progenitor. This has led to the suggestion that cell fates are determined by environmental cues rather than by lineage. In retinas with protracted development, these cues are presumed to change over time since clonal size and composition vary as the retina develops. To test this hypothesis we are studying cell fates after heterochronic transplantation of progenitors in the chick, where the eye is accessible to manipulation at all stages of development.

For transplantation, donor embryos are infected at embryonic day 2 (E2) with a replication-competent retrovirus which allows the virus to spread throughout the embryo. At E6, labelled retinal cells are dissociated and injected into an E3 host chick which is resistant to infection by the marker virus. Isochronic transplants (E6 to E6) are used as controls. Retinas are harvested at E14-16 and transplanted cells are detected in whole mounts using antibody to viral *gag* protein. Results show that clones generated after heterochronic transplants (E6 to E3) are considerably larger than those derived from isochronic transplants.

We are comparing these data with *in situ* lineage analysis of normal retinas. Progenitors are labelled by co-infection with two replication-incompetent retroviruses encoding distinguishable markers. Analysis at E14-16 shows that average clone size is much larger after infection at E3 versus E6.

In conclusion, we have developed a new transplantation protocol that does not suffer from limitations due to interspecies differences and should be of general use throughout the CNS. Using this method in the retina, we have generated data suggesting that the environment can influence the proliferation potential of progenitors.

524.4

STABLE CELL LINES SHOWING NEURONAL PHENOTYPES ESTABLISHED FROM THE MURINE TELENCEPHALON BY TARGETED RETROVIRAL TRANSFORMATION.

Jerold J.M. Chun and Rudolf Jaenisch* Whitehead Institute for Biomedical Research, Nine Cambridge Center, Cambridge, MA 02142

The mammalian CNS exhibits a formidable degree of complexity that makes many molecular biologic issues related to development (e.g. gene expression and function) difficult to approach. The multitude of different cell types at different stages of development is an example of this complexity. In particular, the vast assortment of different neurons - themselves intermixed with non-neuronal cells - makes the study of a homogeneous neuronal population in isolation difficult. Cell lines from different parts of the developing CNS and showing neuronal (rather than mixed non-neuronal) phenotypes could provide a useful reagent to studies of gene expression and function.

We have developed a strategy that appears to target mitotic neuroblasts in the CNS. Since retroviruses require a round of mitosis for proviral integration, and since the "birthdates" of neurons in the cerebral cortex are known, mitotic cells in the ventricular zone could be infected with oncogene-containing retroviruses (similar to lineage studies using LacZ). The results of this approach produced immortalized colonies of neuron-like cells that could not be passaged. Isolation of such colonies followed by reinfection with a different oncogene-containing retrovirus produced rapidly growing transformed cells. Over 30 lines were established by colony expansion (colony selection based on morphology). Four lines were examined in more detail and express all three neurofilaments (200kd, 150kd by Western blot; 68kd by Northern blot; GFAP absent by Northern). Transfection with the 68kd neurofilament promoter driving LacZ or CAT produces expression in the cell lines but not in 3T3 cells. Electron microscopy reveals axon-like processes that appear to contain neurofilaments. Molecular studies using these lines as a tool are in progress.

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524.5

LAMINAR COMMITMENT OCCURS PRIOR TO MIGRATION IN THE DEVELOPING CEREBRAL CORTEX. S.K. McConnell and C.E. Kaznowski*, Dept. of Biological Sciences, Stanford University, Stanford, CA 94305.

The nervous system is populated by an astonishing variety of neurons, differing in their connections, morphologies, and locations. We are studying the generation of this phenotypic diversity in the mammalian cerebral cortex. Each cortical layer contains neurons with similar morphologies, axonal connections, and birthdays. The relationship between cell birthday and adult fate has suggested that newly postmitotic neurons may already be committed to sitting in a certain layer and forming specific axonal projections at the time of their final mitotic division. We are using heterochronic transplantation techniques in ferrets to determine the time at which embryonic neurons become committed to their normal deep-layer fates. Neurons normally destined for layers 5/6 are labeled with [^{3}H]thymidine during S-phase and removed at various times after the pulse. These cells are then transplanted into older host brains, in which upper-layer neurons are being generated; many transplanted neurons migrate into the foreign cortical plate. Our experiments show that young cortical neurons become committed to their normal laminar fates by the time of their terminal mitotic division. If cells are transplanted shortly after [^{3}H]thymidine labeling, when many cells are still in S-phase, transplanted neurons migrate to both the deep and upper layers; thus they represent a mixture of committed and multipotent cells. However, when cells are transplanted 24 hours after labeling, to allow all cells to go through their terminal division in the donor environment, transplanted neurons adopt positions exclusively in the deep layers of the host cortex. Thus, shortly after their final division, all the neurons that migrate display a commitment to their normal fate. We hope to pinpoint the time that commitment occurs by comparing transplant results at different intervals to the dynamics of the cell cycle in the embryonic cortex.

This evidence is consistent with the notion that neuronal progenitors are multipotent, and can be influenced by environmental cues at the time of mitosis to produce a variety of neuronal phenotypes. However, by the time of their final mitosis in the ventricular zone, their progeny become committed to adopting a particular laminar position within the cortex. Laminar commitment thus occurs prior to neuronal migration into the developing cortical layers. (Supported by grants from NIH, Pew Scholars, Searle Scholars, and Clare Boothe Luce Foundation.)

524.6

SEPARATE PROGENITOR CELLS GIVE RISE TO NEURONS, ASTROCYTES AND OLIGODENDROCYTES IN THE RAT CEREBRAL CORTEX J.A. Barfield*[1,2], J.G. Parnavelas[3] and M.B. Luskin[1,2]. Depts. of Anatomy & Cell Biology[1] and Pediatrics[2], Emory Univ. Sch. of Med., Atlanta, GA 30322 and Dept. of Anatomy & Developmental Biology[3], University College London, London WC1E 6BT, U.K.

We have undertaken a study to identify the ultrastructural phenotype of individual cells composing a clone in the rat cerebral cortex using a recombinant retrovirus containing the gene for E. coli ß-galactosidase (*LacZ*) as a lineage tracer. In order to introduce the retroviral marker into precursor cells of the telencephalic ventricular zone, from which all the neurons and macroglia of the cortex originate, recombinant retrovirus was injected into the lateral ventricles of rat embryos on the 15th and 16th days of gestation (corresponding to the onset of neurogenesis). Vibratome sections of adult cortex were incubated with X-Gal to reveal LacZ(+) cells histochemically and processed for electron microscopy. Camera lucida drawings were made to document the position and morphology of LacZ(+) cells and to reconstruct clones. However, we relied on well-established ultrastructural criteria for definitive cell-type determination of serially sectioned LacZ(+) cells; the blue reaction product observed in cells with light microscopy is electron dense under the electron microscope.

When we considered discrete groups of LacZ(+) cells to be clonally related, that is, derived from a single precursor, virtually all of the clones examined contained cells of a single phenotype; they were composed of all neurons, all astrocytes or all oligodendrocytes. Some clones of astrocytes we encountered occurred entirely in the gray matter or white matter, while others extended across both zones. In conclusion, our results provide evidence that the lineages for cortical neurons, astrocytes and oligodendrocytes have diverged by the onset of neurogenesis.

524.7

LINEAGE RELATIONSHIPS OF PYRAMIDAL AND NONPYRAMIDAL NEURONS IN THE RAT CEREBRAL CORTEX. J.G.Parnavelas[1], J.A.Barfield*[2] and M.B.Luskin[2]. [1]Dept.Anatomy, Univ. College London, U.K., & [2]Depts. of Anatomy & Cell Biol. and Pediatrics, Emory Univ., Sch. Med., Atlanta, GA.

In our previous abstract (Barfield et al., 1990) we provided ultrastructural evidence that neuronal and glial lineages in the rat cerebral cortex diverge by embryonic day 15(E15). Here we sought to investigate whether the two principal classes of cortical neurons, the pyramidal and nonpyramidal cells, originate from a common precursor cell. For this purpose, recombinant retrovirus containing the gene for E.coli β-galactosidase (*LacZ*) was injected into the telencephalic ventricles of rat embryos on E15 or E16. Vibratome sections of adult cortex were histochemically stained for LacZ and processed for electron microscopy. The positions of β-Gal(+) cells were mapped to determine in reconstructions which neurons constitute a clone. Clonally related β-Gal(+) neurons were serially sectioned and examined with the electron microscope to ascertain their morphological phenotype.

We used nuclear, cytoplasmic and synaptic features to classify cells; pyramidal neurons have exclusively symmetrical axosomatic synapses, while nonpyramidal cells have both symmetrical and asymmetrical synapses. The clones encountered contained 2-8 neurons and, with one exception, were composed of either all pyramidal or all nonpyramidal neurons. These observations suggest that pyramidal and nonpyramidal neurons are derived from different precursor cells in the ventricular zone.

524.8

ABSENCE OF PERINEURIAL SHEATH AND ASSOCIATED CELLS IN TWIST, A MESODERMLESS MUTANT OF Drosophila melanogaster. J.S. Edwards, L.S. Swales* and C.M. Bate*. Zoology Dept., Cambridge Univ., Cambridge, UK CB2 3EJ.

Both the extracellular neural lamella, which sheaths the CNS, and its underlying cell layer, the perineurium, fail to develop in Drosophila embryos which are homozygous for a mutation in the gene twist (twi^{1d96}/twi^{1d96}). In contrast, the perineurial bracelet cells which underlie the perineurium and which constitute the permeability barrier, appear normal at the ultrastructural level, as do neurons and neuropile glia.

Twist function is required for gastrulation. In loss of function mutants mesodermal cells fail to segregate and all structures of mesodermal origin are thus absent (Nüsslein-Volhard et al., 1984).

The apparent derivation of perineurial sheath cells (perineurium), from mesodermal precursors distinguishes them from neurons and other non-neuronal components of the CNS, all of which are ectodermal in origin. This finding supports the exclusion of the perineurium from the major categories of glia, as proposed by Strausfeld (1976) on anatomical grounds.

Nüsslein-Volhard, C., E. Wieschaus and H. Kluding 1984 Roux's Arch. Devel. Biol. 193, 267-282.

Strausfeld, N.J. 1976 Atlas of an Insect Brain Springer-Verlag.

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524.9

PROPERTIES OF THE SERUM GLYCOPROTEIN(S) THAT INDUCE RAT CEREBRAL TYPE 2 ASTROGLIA FROM BIPOTENTIAL GLIAL PROGENITORS. S.W. Levison and K.D. McCarthy. Curriculum in Neurobiology and Department of Pharmacology. The University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599.

Bipotential glial precursors known as O-2A progenitors become type 2 astroglia rather than oligodendroglia when cultured in fetal bovine serum (FBS) supplemented medium. We have characterized and partially purified an astroglia inducing molecule (AIM) from FBS. An ELISA for glial fibrillary acidic protein (GFAP) and double label immunofluorescence for GFAP and the cell markers A2B5 and ganglioside GD_3 were used to assess AIM's effects on process-bearing cerebral glia. A 200 fold enriched AIM preparation was prepared by precipitating AIM from FBS with 45% ammonium sulfate followed by sequential chromatography on heparin agarose, lentil lectin, and a Superose 12 FPLC sizing column. AIM has a minimum molecular mass of 50 kDa based on gel filtration; however, we have also observed 100 kDa and 440 kDa forms. It is possible that these higher molecular mass AIMs are aggregates of the 50 kDa form since aggregation is observed after exposure to low pH. Alternatively, multiple AIMs may exist. AIM does not appear to be a heparin binding growth factor (HBGF) since it has a higher molecular mass and a lower affinity to heparin agarose than other HBGFs, and its activity is not potentiated by heparin. AIM appears to have an isoelectric point greater than pH 9; however, proteins with a PI near pH 4 also increase the percentage of astroglia in our cultures. To date GFAP inducing activity has not been observed using known serum glycoproteins suggesting that AIM is a novel differentiation factor. (Supported by MH 9828 and NS 20212).

524.10

ROLE OF BRAIN-DERIVED NEUROTROPHIC FACTOR (BDNF) AND NERVE GROWTH FACTOR (NGF) IN THE COMMITMENT OF PLURIPOTENT NEURAL CREST CELLS. M. Sieber-Blum. Dept. of Anatomy and Cellular Biology, Medical College of Wisconsin, Milwaukee, Wisconsin 53226.

The influence of BDNF and NGF on the expression of traits specific for sensory and autonomic neurons by cloned neural crest cells was examined. Pluripotent neural crest cells in clonal culture give rise to melanocytes, adrenergic cells, sensory neuroblasts and to as yet unidentified cells. The mechanisms that lead to these phenotypic restrictions are largely unknown. Neural crest cells at clonal density were grown in the presence or absence of BDNF (10 ng/ml) and/or NGF (25 ng/ml) on a collagen-laminin-fibronectin substratum. All cells within a colony were analyzed by triple labeling, using antibodies against the stage-specific embryonic antigen-1 (SSEA-1) to identify cells in the sensory neuron lineage and against dopamine-β-hydroxylase to visualize adrenergic (autonomic) cells, as well as Hoechst nuclear stain H33258 for total cell counts. In a typical experiment, addition of BDNF caused a 22-fold increase in the number of $DBH^-/SSEA\text{-}1^+$ (sensory) cells (p=0.0001), addition of NGF caused a 4.9-fold increase (p=0.10), whereas in the presence of both BDNF and NGF, a 28-fold increase was observed (p=0.03). The total number of cells per colony did not differ significantly under the various conditions (p=0.82 to 0.89). A newly observed, rare (0.06-0.29%) cell type, $DBH^+/SSEA\text{-}1^+$, expressed both autonomic and sensory traits. These cells developed only in the presence of NGF and/or BDNF and were most numerous when both factors were present. $DBH^+/SSEA\text{-}1^-$ (autonomic) cells developed exclusively in the presence of NGF. Preliminary data indicate that the embryo contains a laminin-dependent cell type, in the presence of which virtually all unpigmented crest cells in clonal culture express SSEA-1, even in the absence of added BDNF and NGF. The results suggest a) that BDNF directs pluripotent neural crest cells or their immediate progeny to differentiate along the sensory neuron lineage, b) that NGF has a similar but less pronounced effect, c) that the stimulatory effect of both factors most likely is additive, d) that NGF may be required for directing pluripotent crest cells to develop along the autonomic cell lineage, and e) that a neural tube- or neural crest-derived cell type produces a strong signal that supports the formation of cells developing along the sensory neuron lineage. Supported by USPHS grant HD21423 and a research grant from the Dysautonomia Foundation.

524.11

CATECHOLAMINERGIC (CA) DIFFERENTIATION IN EMBRYONIC RAT CRANIAL SENSORY GANGLIA: POSSIBLE ROLE OF NERVE GROWTH FACTOR (NGF). D. M. Katz and M. I. Erb*, Depts. of Medicine and Neuroscience, Case Western Reserve Univ. Sch. of Med., Cleveland, OH 44106.

Tyrosine hydroxylase (TH) is transiently expressed by a large subpopulation of nodose and petrosal ganglion (NPG) cells between embryonic days (E) 11.5 and 15.5 in vivo (Jonakait, et al., 1984; Katz and Erb, 1990). In contrast, TH cells are only rarely observed in other cranial sensory ganglia, such as the jugular-superior ganglion (JSG) of the glossopharyngeal and vagal nerves. Consequently, we asked whether or not this difference was due to the absence of cells with CA potential in the JSG. Although mechanisms regulating transient TH expression in embryonic sensory ganglia are unknown, we recently found that NGF treatment of embryonic day (E) 13.5-14.5 NPG neurons in culture increases the proportion of TH cells. In the present study, therefore, we examined whether exposure of JSG cells to NGF in culture would stimulate detectable expression of TH in ganglion cell bodies. Explant and dissociated cultures of E13.5-14.5 JSG were grown for 24 hours in the presence or absence of 25 ng/ml NGF and monitored for TH and neurofilament (NF) protein expression by immunocytochemical staining. In contrast to the JSG in vivo and in control cultures, at least 20% of NF+ ganglion cells in NGF-treated cultures were TH+, indicating the presence of NGF-responsive neurons or neuroblasts with CA potential. These findings raise the possibility, therefore, that the ganglion- specific pattern of transient TH expression observed in vivo may reflect regional differences in extrinsic regulatory influences, such as NGF. Supported by HL-42131 (DMK).

524.12

EFFECTS OF GROWTH FACTORS ON NEURONAL DIFFERENTIATION BY NEURAL CREST CELLS. M.J. HOWARD, K.A. HEIDENREICH, T.P. ROTHMAN AND M.D. GERSHON. DEPT. ANAT/CELL BIOL. COLUMBIA UNIV. NEW YORK, NY 10032 AND DEPT. OF MED. UCSD, LA JOLLA, CA 92092

Cells of the neural crest give rise to diverse derivatives. In tissue culture, the types of neurons that differentiate can be affected by components of the growth medium. Factors in 11 day chick embryo extract (CEE) will support adrenergic differentiation by some neural crest cells in-vitro. The present studies were undertaken to characterize further, responses to the CEE-derived factor and test the possibility that this factor might be insulin. For all studies in which the effect of putative growth factors was being tested, the growth medium was supplemented with 2% CEE, conditions that do not support adrenergic differentiation. When the medium was supplemented with 1-20ng/ml insulin no adrenergic differentiation was detected in trunk neural crest cells after 7 days in culture. Photoaffinity labelling using iodonated B2-2-nitro, 4-(azidophenyl acetyl)-des-PHE^{B1}-insulin, a photoreactive analogue of insulin, failed to detect insulin receptors in membranes prepared from trunk neural crest cells, however, a large number of receptors were labelled in membranes from primary neuronal cultures of E7 chick forebrain. In contrast to the absence of effects with insulin, TGF-β (0.5-5ng/ml) supported adrenergic expression; serotonergic differentiation was enhanced as well. Both arborization of neurites and expression of neurotransmitter related enzymes were enhanced. This is the first characterized growth factor that can substitute in tissue culture for CEE-derived signals that are required for adrenergic differentiation by neural crest cells. The ability of crest cells to respond to CEE-derived factors appears to be transient since cells maintained under restrictive growth conditions for at least 4 days do not show adrenergic differentiation when moved into permissive conditions. In contrast, some crest cells will develop along an adrenergic lineage if they are first exposed to 10% CEE-containing medium for 2-3 days and then transferred to medium containing 2% CEE. Some crest-derived cells will thus express an adrenergic phenotype in response to TGF-β but not to insulin. For CEE-derived factors there appears to be a critical time during which the adrenergic phenotype becomes specified.

CALCIUM CHANNELS IV

525.1

AUTO INHIBITION OF NOREPINEPHRINE RELEASE IN SYMPATHETIC NEURONS IS DUE TO CALCIUM MODULATION AT THE NERVE TERMINAL NOT AT THE CELL BODY. D. Przywara, S. Bhave, A. Bhave*, T. Wakade* & Arun R. Wakade. Dept. of Pharmacology, Wayne State Univ. School of Medicine, Detroit, MI 48201.

We examined the mechanism of inhibition of ^{3}H-norepinephrine (^{3}H-NE) release in cultured neurons from the superior cervical ganglion (SCG) of newborn rats. Electrically evoked (2Hz for 60 sec) release of neuronal ^{3}H-NE was blocked (76±7% maximum decrease) by NE (5 to 50 μM). Similar results were obtained with epinephrine (EPI). The α-2 adrenergic agonist, clonidine (10 μM) had no effect on stimulation evoked ^{3}H-NE release. Whole cell voltage clamp was used to determine the effects of adrenergic agonists on Ca^{2+}-current (I_{Ca}). NE and EPI had no effect on I_{Ca} when ATP or ATP + GTP were included in the recording pipette solution. NE (and EPI) produced only 15% decrease in I_{Ca} when GTP alone was included. Intracellular free Ca concentration ($[Ca^{2+}]_i$) was measured in cell bodies and nerve terminals using Indo-1 fluorescense technique. NE and EPI had no effect on the stimulated (2Hz for 5 sec) rise of $[Ca^{2+}]_i$ in the cell bodies. However, NE and EPI significantly depressed the stimulated rise of $[Ca^{2+}]_i$ in several, but not all, of the nerve terminals. We conclude the effects of NE and EPI on are selectively mediated on the terminal regions of sympathetic neurons, but not on the cell bodies.

525.2

CALCIUM CURRENT OF SYNAPTIC TERMINALS OF GOLDFISH RETINAL BIPOLAR CELLS. Gary Matthews and Ruth Heidelberger, Dept. of Neurobiology, SUNY, Stony Brook, NY 11794-5230

Retinal bipolar cells are non-spiking interneurons that relay information from the photoreceptors to the amacrine and ganglion cells. In goldfish, one class of bipolar cell has a large, bulbous synaptic terminal (~8 μm in diameter) that is well-suited for physiological study. Previously (1990, *Invest. Ophth. Vis. Sci.*, 31, 389), we have used fura-2 to show that depolarization elicits Ca-influx in these cells via dihydropyridine-sensitive Ca-channels. Here, we present a patch-clamp characterization of the Ca-currents in single synaptic terminals. All experiments were done on bipolar cells acutely isolated from adult goldfish retina after papain treatment. Whole-cell Ca-currents were isolated using Cs/TEA pipette solution. From a holding potential of −60 mV, depolarization positive to about −30 mV elicited sustained inward currents that peaked at about 0 mV. There was no transient current component, even with hyperpolarizing prepulses. Removing [Ca]$_o$ abolished the inward current, while isotonic Ba greatly enhanced it. The current was blocked by nitrendipine (0.1 μM) and Cd (0.2 mM) and potentiated by Bay K 8644 (0.1 μM). Currents in somata and terminals were compared, either by severing the axon or by local superfusion of Cd on the terminal or soma. There was no qualitative difference between somatic and terminal Ca-currents, but the terminal accounted for 50–90% of the whole-cell current. Ca-current washed out during whole-cell recording, usually disappearing within a few hundred sec. Wash-out was prevented by leupeptin (Chad & Eckert, 1986, *J. Physiol.*, 378, 31). Indeed, with 0.2 mM leupeptin in the pipette, Ca-current *increased* progressively over the first 60–100 s following break-in, suggesting that Ca-channels in the terminal are subject to regulation. Supported by the National Eye Institute (EY03821).

525.3

THE CALCIUM CURRENT IN THE PRESYNAPTIC NERVE TERMINAL OF THE CHICK GIANT SYNAPSE IS INSENSITIVE TO THE DIHYDROPYRIDINE NIFEDIPINE E.F. Stanley and Aisar H. Atrakchi, LB NINCDS, Bld. 9 Rm 1E124, NIH, Bethesda MD 20892.

We have used the cholinergic chick ciliary ganglion calyx-synapse to test the effect of the DHP, nifedipine, on Ca current (I_{Ca}) recorded directly from a presynaptic nerve terminal (c.f. Brain Res. 505:341). We first used the whole-cell voltage clamp technique in a control neuron to define the holding potential (-50 mV) at which L-type calcium channels are blocked by 10 μM nifedipine. We then used the same conditions to test the effect of the DHP on I_{Ca} recorded from the presynaptic calyx terminal. Locally applied nifedipine did not reduce the calyx I_{Ca}, neither did it block chemical transmission through the ganglion. We conclude that the predominant calcium channel in this presynaptic nerve terminal is not DHP-block sensitive and, hence, can not be characterized as L-type.

525.4

DIFFERENTIAL BEHAVIOR OF SOMA AND GROWTH CONE OF SYMPATHETIC NEURON. Arun R. Wakade, S. Bhave, A. Bhave*, T. Wakade* & D. Przywara. Dept. of Pharmacology, Wayne State Univ. School of Medicine, Detroit, MI 48201.

Neuronal cell bodies have become popular structures to study Ca^{2+} homeostasis by applying the techniques of electrophysiology and fluorescence microscopy. Conclusions derived from these studies are used to explain the role of Ca^{2+} in transmitter release. We demonstrate that Ca^{2+} is handled differently in the cell body and the growth cone, and that changes in Ca^{2+} in the cell body are not reflected in release of ^{3}H-norepinephrine (^{3}H-NE) from cultured sympathetic neurons. Electrical stimulation (10 pulses at 2Hz) produced an almost equal rise in Ca^{2+} in the cell body and growth cone, but it's removal was much faster and complete in the growth cone. Substitution of Ca^{2+} by Ba^{2+} enhanced current in the cell body but not in the growth cone. Caffeine (10 mM) enhanced cell body Ca^{2+} but did not change growth cone Ca^{2+} levels and did not increase ^{3}H-NE release. Although cadmium and verapamil (50 μM) completely blocked Ca^{2+} transport in the cell body these agents produced only partial inhibition (60-73%) of ^{3}H-NE release even when used in high concentrations (200 μM). We conclude that examination of Ca^{2+} movements only in the cell body is insufficient to understand the role of Ca^{2+} in the release of transmitter and it's modulation by pharmacological agents.

525.5

DISTRIBUTION OF CALCIUM CHANNELS ALONG FROG MOTOR NERVE TERMINALS. M.W. Cohen, O.T. Jones* and K.J. Angelides. McGill Univ., Montreal, Que. and Baylor Col. of Med., Houston, TX.

Tetramethylrhodamine-conjugated ω-conotoxin (TRωCT; Science 244 1189, 1989) was used to examine the distribution of voltage-gated calcium channels on motor nerve terminals in the sartorius muscle of Xenopus laevis. In most experiments acetylcholine receptors were also stained with fluorescein-conjugated α-bungarotoxin. TRωCT blocked neuromuscular transmission and stained neuromuscular junctions faintly. In face views the fluorescence consisted of discrete transverse bands at intervals of about 1 μm along the length of the synapse. In side views it consisted of discrete dots, also at 1 μm intervals. This staining was not observed when muscles were pretreated with ωCT or when the motor nerve terminals were made to degenerate by nerve resection. Additional observations indicated that the TRωCT staining was restricted to the synaptic side of the nerve terminals and aligned with the junctional folds. It is concluded that voltage-gated calcium channels on frog motor nerve terminals are clustered at active zones. This distribution ensures that during the presynaptic action potential intraterminal Ca^{++} reaches its peak concentration at the same sites where synaptic vesicles are clustered. (Supported by MRC of Canada and by NIH).

525.6

MEASUREMENT OF INTRACELLULAR CALCIUM IN CULTURED PURKINJE CELLS UNDER VOLTAGE CLAMP. M.H. Dickinson and J.A. Connor. Dept. of Neurosciences, Roche Institute of Molecular Biology, 340 Kingsland St., Nutley, NJ 07110.

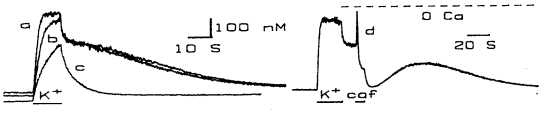
Traditional methods of whole cell patch recording which disrupt endogenous buffering systems have made it difficult to study calcium localization in voltage clamped neurons. By using the Nystatin perforated patch technique, we have been able to voltage clamp the somata of cultured Purkinje cells while simultaneously imaging intracellular calcium in both soma and dendrites with the indicator fura-2. Using this approach, we are examining the spatial and temporal changes in calcium concentration caused by alteration of membrane potential and iontophoretic application of excitatory amino acids.

Glutamate stimulation of Purkinje cells causes a transient calcium influx with components sensitive to both nifedipine and APV (Hockberger, et al., J. Neurosci. 9: 2272, 1989). Repetitive stimulation can result in maintained elevations of intracellular calcium that long outlast the glutamate pulses. Such elevations might be involved in tonic alterations of Purkinje cell membrane properties, such as those produced during Long Term Depression. We are using the simultaneous voltage clamp-imaging technique to determine the relative contributions of the voltage and ligand gated conductances in both the transient and maintained calcium responses.

525.7

TWO PHARMACOLOGICALLY DISTINCT Ca^{2+} STORES WHICH MODIFY $[Ca^{2+}]_i$ ELEVATIONS PRODUCED BY DEPOLARIZATION IN SYMPATHETIC NEURONS D.D. Friel and R.W. Tsien, Department of Molecular and Cellular Physiology, Stanford Univ. School of Medicine, Stanford, CA 94305.

We studied effects of intracellular compartments on $[Ca^{2+}]_i$ responses elicited by 50 mM K^+ in bullfrog sympathetic neurons, focusing on contributions from two stores, a ryanodine (ryan) and a caffeine (caf)-sensitive store (RSS) and a ryan and caf-insensitive store (RIS). Either store appears to act as a Ca source or sink depending on its Ca content and $[Ca^{2+}]_i$. Ryan was used to study the ability of the RSS to act as a Ca source. Responses elicited in the presence of 1 μM ryan (b), which inhibits caf responses, were slower in onset but otherwise similar to controls (a), suggesting that the K^+-induced $[Ca^{2+}]_i$ rise is normally speeded by Ca release from the RSS. Caf was used to transform the RSS into a Ca sink. When K^+ was applied (in the absence of caf) shortly after a caf-induced $[Ca^{2+}]_i$ transient, $[Ca^{2+}]_i$ rose slowly (c), in contrast to the rapid $[Ca^{2+}]_i$ rise elicited in the continued presence of caf. One explanation for the slow onset (and fast recovery) in (c) is that under these conditions the RSS behaves as a Ca sink. The other store (RIS) appears to prolong recovery by releasing Ca that it accumulates when $[Ca^{2+}]_i$ is elevated, producing a ryan-insensitive plateau (compare a,b). If caf is applied briefly during the plateau (d), $[Ca^{2+}]_i$ rises transiently; after caf is removed, $[Ca^{2+}]_i$ falls and then undergoes a second transient rise, even in the absence of external Ca, suggesting that the plateau arises from release of Ca from an internal source. In the presence of 1 μM FCCP, K^+ elicits larger $[Ca^{2+}]_i$ responses with no obvious plateaus, suggesting that Ca uptake and release by this store is sensitive to FCCP. Acting together, the RSS and the RIS may shape stimulus-evoked $[Ca^{2+}]_i$ transients in a way that reflect the cells' history of stimulation.



525.8

SINGLE CHANNEL BEHAVIOR OF L-TYPE CALCIUM CHANNELS DURING CALCIUM-DEPENDENT INACTIVATION. R.H. Kramer & E.S. Levitan HHMI and Columbia U., NY, NY 10032, and Dept. of Pharmacol., Yale U. Sch. of Med., New Haven, CT 06310.

The L-type Ca current in many cells is inactivated by intracellular Ca^{2+}, but the single-channel basis for this is not known. We are using the perforated patch technique to study Ca-dependent inactivation of single L channels in pituitary tumor cells (GH3). In all experiments BAY K 8644 was present to accentuate L channels. First, we recorded single outside-out L channels in a nystatin-perforated excised vesicle, replete with intact second messenger systems and organelles. Application of the neuropeptide TRH inactivates L channels in the vesicle (5 of 8 tries). TRH decreased opening, without affecting single channel conductance (γ). In two analysed cases, mean open time decreased by about 50%. Evidence strongly suggests that TRH generates IP3 which mobilizes internal Ca^{2+}, thereby inactivating L channels. The TRH-induced rise in Ca^{2+} is coincident with inactivation and is both necessary and sufficient; e.g. the TRH effect is blocked by EGTA, while activators, inhibitors, and down-regulation of protein kinase C have no effect, and many other agents that release internal Ca^{2+} also inactivate L channels.

Second, we used a double patch configuration: one pipette for whole-cell perforated voltage-clamping the cell, the second for cell-attached recording of Ba^{2+} current through single L channels. Depolarizing the cell reversibly decreases channel opening without affecting γ, and the inactivation is Ca-current dependent. These experiments provide a direct observation of Ca-dependent inactivation of Ca channels in intact cells.

525.9

PHYSIOLOGICAL MODULATION OF Ca2+ CHANNELS IN THE LIVING HUMAN BRAIN STUDIED WITH P.E.T.

P.E.Roland, G. Blomqvist, R.J. Seitz, S.Stone-Elander, E Schwenner#, H.Kraft#, C. Halldin, H. Böshagen#. PET section, Karolinska Institute and Hospital, S10401 Stockholm Sweden. # BAYER Wuppertal. FRG.

Ion channels in mammalian brains have so far only been studied in vitro. Under in vivo conditions we thought that the 1,4-dihydropyridine nimodipine would show use-dependent binding to the Ca^{2+} L-channel. (11)C-nimodipine was injected in normal volunteers performing a somatosensory discrimination task (Roland, *Arch. Neurol.* 44: 543, 1976) while the fate of the tracer in the brain was measured with a PET camera. In another group of volunteers the regional cerebral blood flow (rCBF) was measured during the same somatosensory discrimination task and during rest. The rCBF increases marked the cerebral structures participating in the task. The maximal binding capacity, Bmax, of (11)C-nimodipine was calculated from differential equations describing a three compartmental model (Blomqvist et al. 1990). Injections of cold nifedipine and nimodipine demonstrated that the specific binding originated from the brain cells and not the vasculature. Bmax was in the average 2.3 pM/cc in un-stimulated cortex, but 33- 68% higher in the anatomical structures participating in the task: contralateral ventral thalamus, sensory-motor cortex, putamen-pallidum (bilat.) and ipsilateral ant. lobe of cerebellum. The results demonstrate use dependent binding of nimodipine **in vivo** and that the L-channel activates during physiological brain work in the structures participating in the task.

525.10

STUDY OF CALCIUM CHANNELS IN ACUTELY ISOLATED CA3 HIPPOCAMPAL PYRAMIDAL NEURONS SUGGESTS FOUR DIFFERENT TYPES D.J. Mogul and A.P. Fox* Dept. of Pharmacology and Physiology, University of Chicago, Chicago, IL 60637

Pyramidal neurons from the CA3 region of young adult guinea pig hippocampus were enzymatically dissociated (Kay & Wong, 1987). Cells were voltage-clamped using both the whole-cell and single-channel configurations. Three of the channels resembled T-, N-, and L-type channels seen elsewhere. These channels had unitary conductances of 7 pS, 12 pS, and 23 pS, respectively. T channels activated at test potentials (TP) between -60 and -50 mV, showed rapid inactivation, were blocked by 100 μM Ni^{2+} and 500 μM amiloride, and showed resistance to 50 μM Cd^{2+} block. N channels required negative holding potentials (HP) to reprime, activated when TP > -30 mV, and were blocked by 1 μM w-CgTx. L channels showed sensitivity to dihydropyridine agonists (BayK 8644) and antagonists (nimodipine) and displayed negligible inactivation during a test pulse. However, certain differences were found in these channels compared to previous reports. T channels showed a slower inactivation rate and required a very negative holding potential to be reprimed ($V_{1/2}$~-110 mV). L-type Ca^{2+} channels were suppressed by 2 μM nimodipine (NIM), from HP = -50 mV. In contrast, from HP = -90 mV, NIM augmented the L-type currents elicited by TP between -50 and -20 mV and shifted the peak of the I-V in a manner similar to BayK 8644 (1 μM). In addition to these differences, a high-threshold whole-cell current component was present in these cells that was not blocked by NIM or w-CgTx at any concentration. No direct measurement of unitary current for this component has yet been recorded suggesting that its conductance is either very similar to one of the other three channels or it is situated outside the soma where the cell-attached patches were taken.

525.11

CALCIUM CHANNEL SUBTYPES IN ACUTELY ISOLATED ADULT RAT AND FROG SENSORY NEURON SOMATA. R.S. Scroggs and A.P. Fox. Department of Pharmacology and Physiology, University of Chicago, 60637

Dihydropyridines (DHP) and omega-conotoxin GVIA (ω-CgTx) were tested on Ca^{2+} currents recorded from acutely isolated rat and frog dorsal root ganglion cells. Bay K 8644 increased peak current in rat cells by 56% ± 16.2 SE (N=5). Sequential treatment with 2μM nimodipine and then 1μM ω-CgTx, blocked different portions of the total current in rat and frog cells. ω-CgTx was more effective, nimodipine less effective, and more current was left unblocked in cells held at -80mV versus -60mV (Table below).

Species	Holding Potential	% Decrease 2uM Nimod	% Decrease 1μM ω-CgTx	% Current Remaining	N
Frog	-60mV	50 ± 4.8 SE	47 ± 4.0 SE	2 ± 1.8 SE	7
Frog	-80mV	14 ± 3.4 SE	71 ± 3.7 SE	12 ± 2.0 SE	6
Rat	-60mV	64 ± 4.1 SE	20 ± 2.7 SE	8 ± 2.0 SE	5
Rat	-80mV	26 ± 4.0 SE	43 ± 3.6 SE	22 ± 2.6 SE	10

The increase in ω-CgTx induced block at negative holding potentials may reflect an increase in the number of primed N channels. Since changing the holding potential from -60mV to -80mV increased peak current by 80% (N=6), the proportion of current blocked by nimodipine at -80mV is less than the expected (36% decrease in rat, 28% decrease in frog) based on the effect of nimodipine observed at -60mV. Thus, the unblocked current may reflect a decrease in efficacy of DHP antagonists on L channels at negative holding potentials. More unblocked current was observed in rat than frog which may reflect the presence of DHP and ω-CgTx resistant channels in the rat cells.

525.12

INACTIVATION PROPERTIES AND PHARMACOLOGY DISTINGUISH TWO CALCIUM CURRENTS IN CULTURED MOUSE PANCREATIC ß-CELLS. W.F. Hopkins, L.S. Satin, and D.L. Cook*, Depts. of Physiology/Biophysics and Medicine, Univ. of Washington and VA Medical Center, Seattle, WA 98108.

We have shown that two types of insulin-secreting cells (neonatal rat ß-cells and HIT cells) each possess two calcium currents (Satin and Cook, *Pflügers Arch.* **411**: 401-409, 1988; *Pflügers Arch.* **414**: 1-10, 1989). In contrast, mouse ß-cells have been reported to possess only L-type calcium channels. To reexamine this question, we studied calcium currents in adult mouse ß-cells using the whole-cell voltage clamp technique. When calcium currents were elicited with 10 sec test pulses, the time course of inactivation was well fit by the sum of two exponentials. The fast component's time constant was 75 ± 5 msec (at 0 mV) and it displayed calcium- and voltage-dependent inactivation, while the slow component's time constant was 2750 ± 280 msec and it inactivated primarily via voltage. The fast component showed greater steady-state inactivation at holding potentials between -100 and -40 mV and had a lower voltage threshold than the slow component. Nimodipine (0.5 μM) blocked 43 ± 4% of the fast component (from -100 mV) but had no effect on the slow component. Higher doses of nimodipine (≥ 1 μM) decreased both components. The amplitude of the slow component was significantly increased by replacing calcium with barium, while the fast component was not changed. The slow component also displayed a ten-fold greater sensitivity to cadmium block than the low-threshold, fast component. The data suggest that mouse ß-cells, as with other insulin-secreting cells, possess at least two distinct calcium currents.

Supported by NIH grant DK29816 and the Veterans Administration.

mRNA REGULATION: PEPTIDES AND C-FOS

526.1

ESTROGEN (E) INFLUENCES ON OXYTOCIN mRNA EXPRESSION IN PREOPTIC AND ANTERIOR HYPOTHALAMIC REGIONS STUDIED BY IN SITU HYBRIDIZATION. S.K. Chung and D.W. Pfaff, The Rockefeller University, New York, NY 10021.

In order to analyze possible influences of E on oxytocin mRNA expression at preoptic and anterior hypothalamic levels of the rat brain, in situ hybridization was used, supported by immunocytochemistry and compared to vasopressin mRNA in situ hybridization. A tritiated 25 base oligomer was used, previously confirmed as oxytocin-specific (Kawata et al., Brain Res. Bull., 1988). Ovariectomized female rats were treated for 2 days or 2 months with estradiol (10%, mixed in cholesterol) or cholesterol (control) in subcutaneous capsules (5mm length). Oxytocin mRNA in situ hybridization and immunocytochemistry both showed neurons expressing in MPOA, ACN, periventricular stratum, PVN, SON, perifornical nucleus, BNST, NC and intersupraoptico-paraventricular islands. While there was a trend for the number of oxytocin mRNA-containing neurons to increase (about 2X) after 2 days of E treatment, this was not significant. When the amount of oxytocin mRNA per labelled neuron was quantified, E treatment (either 2 days or 2 months) was shown to significantly increase oxytocin expression in SON and ACN, approximately doubling pixels per neuron. The E effect could depend on an ERE identified upstream of the oxytocin gene, but it could also be secondary to changes in release of the peptide.

526.2

TRANSCRIPTION FACTORS BINDING TO THE PREPROENKEPHALIN PROMOTER: EXPRESSION IN NEURONS OF NORMAL AND STIMULATED NUCLEUS CAUDALIS. G.R. Uhl, M.G. Buzzi, D. Appleby*, M.A. Moskowitz and T. Nishimori. Lab. of Mol. Neurobiol., NIDA/ARC and Depts. of Neurol. & Nsci., Johns Hopkins Sch. of Med., Bx 5180, Baltimore, MD 21224 and Dept. of Neurol & Nsurg, MGH and HMS, Boston, MA 02114

Neurons expressing preproenkephalin in lamina I and II of the nucleus caudalis display exquisite activity-related changes in preproenkephalin gene expression. A region of the proenkephalin promoter that may bind and be regulated by a specific set of transcription factors has been identified. To understand possible mechanisms of trans-synaptic preproenkephalin regulation in these neurons, mRNAs encoding these factors were studied in nucleus caudalis neurons from brains sacrificed after unilateral stimulation of the trigeminal nerve using in situ hybridization with matched oligonucleotide cDNA probes.

Levels of hybridization to Jun B mRNA are greater than those for cJun, with only scattered neurons hybridizing with Jun D probes. Expression of Jun B is also greater than that of cFos, AP2, NF1-X and NF1-red1. After primary afferent stimulation, expression of both Jun B and cfos mRNAs is noted in more neurons. These results are consistent with a role for AP-1 factors in the upregulation of proenkephalin noted after primary afferent stimulation.

526.3

SEX STEROIDS REGULATE THE DEVELOPMENT AND ADULT EXPRESSION OF PRO-OPIOID mRNA IN THE ANTEROVENTRAL PERIVENTRICULAR NUCLEUS (AVPv). R.B. Simerly. Oregon Regional Primate Research Center, Beaverton, OR 97006.

Opioid peptides are generally believed to exert important regulatory influences on gonadotropin secretion. Recently (Simerly et al., *J. Comp. Neurol.* **276**:442), a sexually dimorphic population of enkephalinergic cells was identified in the AVPv, a nucleus previously shown to play a critical role in the neural control of ovulation (Weigand & Terasawa, *Neuroenocrinol.*, **34**:395). Neither proopiomelanocortin- nor dynorphin-immunoreactive neurons were found in the AVPv, however, the nucleus appears to contain a discrete population of cells that contain prodynorphin (PDYN) mRNA suggesting that an opioid peptide other than authentic dynorphin may be the predominant peptide derived from PDYN in these neurons. In the present study we used *in situ* hybridization and asymmetric RNA probes synthesized from cDNAs, generously provided by Drs. S. Sabol and J. Douglass, to examine the distribution and hormonal regulation of mRNAs encoding proenkephalin (PENK) and PDYN in the AVPv. As suggested by our previous immunohistochemical results, the AVPv contains more cells that express PENK mRNA in male rats. In contrast to PENK, a greater number of PDYN mRNA-containing neurons are found in female rats, and both of these neurochemical sex differences are determined, at least in part, by perinatal exposure to androgens. In order to evaluate the possible regulation of PENK and PDYN expression within these sexually dimorphic populations of neurons, we measured PENK and PDYN mRNA hybridization in the AVPv of female and male rats that were either intact, or gonadectomized and implanted with pellets of 17β-estradiol (or testosterone) for 1, 3, or 7 days before sacrifice. Within 24 hrs. estradiol treatment increased PDYN mRNA levels by over 60% relative to either intact estrous females, or ovariectomized controls. In contrast, comparable differences in PDYN mRNA levels were not found in male rats, nor were changes in levels of PENK mRNA detected within the AVPv in hormone-treated animals of either sex. Taken together these results suggest that circulating sex steroids differentially influence the development of PENK- and PDYN-containing neurons in the AVPv during the perinatal period, and that estrogen upregulates PDYN gene expression in the adult female rat. Thus, the maintenance of PENK cells, and the loss of hormone-sensitive PDYN cells, in the AVPv may represent important developmental influences of perinatal androgens on the sexual differentiation of gonadotropin secretion. Supported by NIH grant NS2672.

526.4

IN SITU DETECTION OF POMC HETERONUCLEAR RNA IN INDIVIDUAL NUCLEI IN RAT BRAIN AND PITUITARY. M.K.-H. Schafer*, J.P. Herman, R.C. Thompson and S.J. Watson. Mental Health Research Institute, University of Michigan, Ann Arbor, MI 48109.

In- situ hybridization techniques (ISH) using exon coding probes to study the regulation of neuroendocrine peptide mRNA expression are well established. However, measurement of cytoplasmatic mRNA levels are less suitable to determine acute or small changes in biosynthetic activity. In contrast, transcription assays measuring the primary gene transcript in the nucleus appear to reflect the transcriptional rate of the gene much more precisely. Recently, Fremeau et al. (Science, 1986) have succeeded in studying the expression of the POMC gene in individual pituitary cells by ISH using riboprobes specific for intervening sequences (introns). Due to the low abundance of the primary transcript and the long exposure times these studies have been tedious.

In this study we localized POMC hnRNA both in individual cells of the rat arcuate nucleus and the pituitary gland by ISH. Riboprobes specific for Intron A of the POMC primary transcript were labeled either with high specificity ^{35}S or digoxigenin-UTP for non-radioactive detection. Radioactively labeled hybrids could be detected in pituitary nuclei after one week of exposure time and in the arcuate nucleus after one month. The enzymatic detection of the non-radioactive labeled hybrids yielded positively stained cells in the pituitary gland within 24 hours. However, in the arcuate nucleus the latter method failed to produce a detectable signal. While the quantifiablity of the non-radioactive approach needs to be determined, it shows a clear advantage of both excellent morphological resolution and rapid signal detection. At present we are investigating, whether the hnRNA detection by ISH using intron specific probes truelly reflect transcriptional activity.
This work was supported by MH42251, NIDA 022414 and DK07245.

526.5

LOCALIZATION AND REGULATION OF VASOPRESSIN HETERONUCLEAR RNA IN THE RAT HYPOTHALAMUS. J.P. Herman, T.G. Sherman, M.K.-H. Schäfer, and S.J. Watson. Mental Health Research Institute, University of Michigan, Ann Arbor, MI 48198-0720.

We have used a cRNA probe directed against a purely intronic sequence of the rat provasopressin (VP) gene to localize VP heteronuclear (hn) RNA within individual hypothalamic neurons via *in situ* hybridization histochemistry. The sequence used for probe construction was free from known repetitive sequences and yielded a single band upon Southern blot analysis of Sprague-Dawley genomic DNA, confirming its specificity for study of proVP hnRNA. Standard ISHH procedures using this probe yielded a positive signal in hypothalami of normal rats localized to nuclei in the supraoptic, paraventricular and suprachiasmatic nuclei. In contrast, ISHH using probes directed against exon C of proVP showed a primarily cytoplasmic localization of proVP mRNA in these nuclei. No signal was observed in tissue pre-digested with RNAse A or in tissue incubated with sense-strand intron probe. The signal intensity obtained with the intron probe was surprisingly robust, being visible within 24 hours of exposure to Kodak XAR X-ray film and acheiving optimal intensity at 41 hours. Preliminary regulation studies indicate a consistent up-regulation of VP hnRNA in the SON and PVN in response to 4 days of salt-loading (2% saline), suggesting that osmotic stimuli effectively increase hnRNA levels in a long-term manner.

It is generally believed that hnRNA is rapidly processed in the nucleus, with the spliced-out intervening sequences rapidly degraded. As such, levels of nuclear hnRNA may be taken to reflect recent gene transcription. These data suggest that detection of hnRNA by ISHH may represent a technique whereby gene transcription can be studied in anatomically-specified neurons.

Supported by NS 08267 and MH422251.

526.6

EFFECT OF GONADAL STEROIDS ON PITUITARY PRODYNORPHIN mRNA LEVELS IN THE MALE RAT. R. Day, M. Hoversten* and H. Akil. Mental Health Research Institute, University of Michigan, Ann Arbor, MI, 48109-0720.

Prodynorphin (PDYN) is the precursor of a series of well characterized opioid peptides with leucine-enkephalin extended sequences which include dynorphin A 1-17, dynorphin B and α-neo-endorphin. In the rat pituitary, PDYN derived peptide-immunoreactivity (IR) has been detected in the anterior pituitary, co-localized with a subset of gonadotrophs, the cells known to produce LH and FSH. The influence of gonadal steroids on gonadotrophs is relatively well understood. In the male rat, testosterone has an inhibitory feedback on LH and FSH. Recent studies looking at the effect of gonadal steroids on the PDYN peptides, showed that short term castration (3-5 days) lowers PDYN peptide-IR, while long term castration (30 days) increases PDYN peptide-IR and that testosterone reverses this effect. In the present study, we examined the effects of short and long term castration on anterior lobe PDYN mRNA. Male rats (200-225 g) were castrated for periods of 5 days, 14 days or 30 days. In one experiment, testosterone (2x day, 100 µg/100 g body weight, sc) was given for 10 days, 7 days after the castration surgery. Anterior pituitaries were dissected and total RNA was extracted and submitted to Northern gel analysis. A significant increase in PDYN mRNA was observed in rats castrated for 5, 14 or 30 days. Testosterone reversed the effects of castration, returning PDYN mRNA to normal levels. Interestingly, sham-operated rats receiving tetosterone had significantly lower levels of PDYN mRNA than sham-operated rats. Our data indicate that anterior pituitary PDYN is under the inhibitory influence of gonadal steroids and that removal of this feedback mechanism activates its synthetic capacity. Supported by the Theophile Raphael Fund and NIMH grant MH 422251. R.D. is a fellow of the Medical Research Council (MRC) of Canada.

526.7

DIFFERENTIAL EXPRESSION AND DEVELOPMENTAL REGULATION OF TACHYKININ RECEPTOR mRNAS IN THE RAT CENTRAL NERVOUS SYSTEM. J.E. Krause, A.D. Hershey, Y. Takeda, and S.P. Sivam. Department of Anatomy and Neurobiology, Washington University School of Medicine, St. Louis, MO 63110 and Department of Pharmacology and Toxicology, Indiana University School of Medicine, Gary, IN 46408.

We are interested in the functions and mechanisms of action of tachykinin peptides in the rat central nervous system (CNS). Toward this end, we have cloned cDNAs encoding a rat substance P receptor (SPR; NK-1 type) and a rat neurokinin A receptor (NKAR; NK-2 type). In the present studies, we have addressed some issues of expression of these mRNAs in discrete CNS regions and during CNS development. The mRNAs encoding these tachykinin receptors are low abundance species, consequently highly specific and sensitive nuclease protection assays were established for receptor mRNA detection and quantitation. SPR mRNA is widely expressed throughout the adult CNS, with highest level present in striatum, hippocampus and midbrain. SPR mRNA was detected in many other CNS regions, and overall the levels of abundance in CNS regions ranged from 0.001% (striatum) to 0.00008% (cerebellum) of total RNA. NKAR mRNA was detectable only in striatum and hippocampus, and these mRNA levels were at least 100-fold lower than that observed for SPR mRNA. The expression of SPR mRNA is developmentally regulated in whole brain and striatal RNA, with detectable levels present at E14 and levels greater than adult were present from embryonic day 18 to postnatal day 21. Similar studies are currently underway for NKAR mRNA. Overall, these studies demonstrate that 1) SPR and NKAR mRNAs are of extremely low abundance in the CNS, 2) the mRNAs show region specific patterns of expression and 3) they are developmentally regulated.

526.8

REGULATION OF c-FOS EXPRESSION BY VIP AND OTHER ACTIVATORS OF THE CYCLIC AMP SYSTEM IN PRIMARY CULTURES OF RAT CEREBRAL CORTEX. F.M. Vaccarino, M.D. Hayward*, J.F. Tallman, R.S. Duman, and E.J. Nestler. Lab. of Molecular Psychiatry, Depts. of Psychiatry and Pharmacology, Yale Univ. School of Med., New Haven, CT 06508.

The c-fos gene promoter is known to respond to calcium and cyclic AMP, but virtually all studies have focused on activators of the calcium system (e.g., seizures, excitatory amino acids) as inducers of c-fos expression in the nervous system. The present study demonstrates that activation of the cyclic AMP system also induces c-fos expression in brain.

We studied the regulation of c-fos mRNA levels by Northern blotting in primary cultures of rat cerebral cortical neurons. A number of neurotransmitter ligands, known to increase cyclic AMP levels, increased c-fos expression, including VIP, isoproterenol, dopamine, and serotonin. The VIP-induced increase in c-fos was similar in magnitude to that seen in response to glutamate, which was found to induce c-fos via actions at both NMDA and non-NMDA receptors, probably through activation of the calcium system. However, the effect of VIP was not modified by the glutamate receptor antagonists MK-801 and CNQX, indicating that VIP induction of c-fos is not secondary to the release of endogenous glutamate. Forskolin (which stimulates adenylate cyclase) or lipophilic derivatives of cyclic AMP also increased c-fos mRNA levels in these cultures.

Taken together, the data demonstrate that, in differentiated cerebral cortical neurons, diverse types of neurotransmitters, through multiple intracellular pathways, lead to regulation of c-fos expression which could then mediate some of the long-term effects of the neurotransmitters on gene expression.

526.9

WOUNDING OF THE CORNEA LEADS TO THE RAPID EXPRESSION OF C-FOS IN TRIGEMINAL NEURONS. H.W. Thompson 1, S. Griffin 1, R.W. Beuerman. Lions Eye Research Labs and Laboratory of the Molecular Biology of the Ocular Surface, L.S.U. Eye Center, New Orleans, LA 70122.

The cornea has the most densely innervated epithelial surface in the body. Previous studies from this laboratory have shown that removal of a 6mm, 150-200 um thick disc of tissue from the rabbit extirpates free nerve endings and the underlying subepithelial plexus while inducing the formation of a dense ring of collateral sprouts by 24 hrs(Roza, Guss, Beuerman, Invest Ophthalmol Vis Sci 24:1983,1033-1051). Sensory stimulation of free nerve endings in the epithelium around the wound shows a loss of modality specificity, spontaneous activity, and an increase in action potential output to a given stimulus. The present study has investigated gene expression in the soma after wounding by analysis of the mRNAs of the rabbit trigeminal ganglion at 0, 15, 30, 45 min, and 1 hr after wounding. Total cellular RNA was extracted denatured and dotted on nitrocellulose. The polymerase chain reaction (PCR) was use to detect c-fos mRNA and dot blots of RNA and PCR products were hybridized with 32P labelled c-fos plasmid (ATCC #41042). Compared with RNA extracted from cortex of the same animals, and trigeminal ganglia from unwounded animals the level of c-fos mRNA rose to a peak at 15 minutes following wounding, and was in decline by 1 hr. The change in electrical activity following corneal wounding may lead to the increased expression of c-fos message in this sensory ganglia. (EY04074, EY02311)

526.10

ELEVATION OF STRIATAL C-FOS mRNA AND AP1 COMPLEX FORMATION AFTER TREATMENT WITH COCAINE. M.J. Iadarola, C.L. Yeung*, Y. Hoo* and +J.P. Quinn*. Neurobiology and Anesthesiology Branch NIDR and +Laboratory of Pathology, NCI, NIH, Bethesda MD.

Levels of Fos protein and Fos-related antigens are elevated by indirect-acting dopaminergic agonists (e.g. cocaine) acting through D1 receptors. The present experiments address the relationship of protein elevations to c-fos mRNA and the ability of tissue extracts to reconstitute a fos/jun (AP1) complex. RNA blot analysis was performed to measure the levels of c-fos mRNA in caudate, frontal cortex, hippocampus and cerebellum. AP1 complex formation was assessed by gel mobility shift assays using extracts from these brain regions and an oligonucleotide from the gibbon ape leukemia virus enhancer (GALV) which contains the AP1 consensus sequence(TGAGTCA). Cocaine (5 to 40 mg/kg, ip) produced dose-related increases in c-fos mRNA of up to 8 fold within 30 min. The most marked increases were observed in straitum, consistent with its dense dopaminergic innervation. The cerebellum also consistently showed an increase in c-fos mRNA while little or no alteration occurred in frontal cortex or hippocampus. Gel shift analysis showed that all areas contained proteins capable of forming an AP1 complex with GALV. An additional mobility shift of the AP1 complex was consistently observed upon addition of an antibody to c-fos. While the intensity of the AP1 shifts was somewhat greater in striatal extracts from the cocaine treated rats in comparison to saline treated rats, it did not exceed two-fold. These data indicate that cocaine elevates c-fos gene expression in discrete brain regions and further suggest a role for specific Fos proteins in the regulation of neuronal genes containing AP1 sites.

526.11

AMPLIFICATION OF REGULATED mRNA FROM OPIATE-TREATED CELLS. SA Mackler & JH Eberwine. Dept of Pharmacology, Univ. of Pa. School of Medicine, Phila. PA 19104.

We are using amplification of mRNA (aRNA;PNAS 87:1663) to study the effects of opiate stimulation or withdrawal of opiate stimulation on neuronal gene expression. The goal of these studies is to define the molecules underlying opiate tolerance and physical dependence.

In efforts to clone and characterize opiate-regulated mRNAS from discrete brain regions we are using *in situ* transcription-aRNA based differential hybridization. This technique permits us to screen multiple candidate cDNA clones to determine whether they are opiate-regulated using radiolabeled probe made from individusal tissue sections. We have also used these probes in differential hybridization screening of a library.from delta opiate receptor-expressing NG108-15 cells to select several clones which are now being characterized.

We have studied G protein coupled receptors in NG108-15 cells. A degenerate 51mer oligonucleotide was synthesized based upon a conserved sequence of cloned G protein coupled receptors. This 51mer contained a T7 RNA polymerase promoter five prime to the sequence used to prime cDNA synthesis on total RNA isolated from three separate tissues: untreated NG108-15 cells, cells treated with 10nM DADLE for 24 hrs and cells treated with 10nM DADLE for 16 hrs followed by 10uM naloxone alone for 8 hrs. After second strand cDNA synthesis, aRNA was synthesized with 32-P CTP and used to screen rat genomic DNA. Reversal of opiate stimulation resulted in an increase in abundance of RNA hybridizing to a 2.2kB Eco R1 fragment. This aRNA is being used to screen NG108-15, PC12h, and rat striatum libraries.

It is hoped that a more complete understanding of the molecular changes from opiate use will illuminate the mechanisms of addiction.

526.12

REMOVAL OF CRANIAL BONES INCREASES GENE EXPRESSION OF RAT CORTICAL NEURONS D.K. Meyer *, C. Olenik * and J.J. Vanderhaeghen. Dept. Pharmacol., Freiburg University, D-7800 Freiburg, Fed. Rep. Germany and Neuropathol. Res. Lab., Free University, B-1070 Brussels, Belgium.

Removal of cranial bones is a prerequisite for stereotaxic operations. We have observed that this procedure transiently enhances the concentration of mRNAs coding for preprocholecystokinin (CCK-mRNA) and preprosomatostatin in rat cortex (Olenik and Meyer, Neuropeptides, 15: 115, 1990). Both neuropeptides are co-localized with GABA in a subpopulation of cortical interneurons (Jones and Hendry, TINS, 9: 71, 1986).

In the present study, we have investigated where the respective peptide neurons are located and whether the expression of other genes is also changed under these circumstances. In situ hybridization studies showed that the neurons containing CCK-mRNA are mainly located in layers II and III as well as V and VI of rat neocortex. Removal of the right parietal bone enhanced the labeling of these layers, but only of the ipsilateral side.The density of CCK-mRNA was not changed in subcortical areas. Thus, the phenomenon seemed to be restricted to the ipsilateral cortical areas. The activity of glutamate decarboxylase, the rate limiting enzyme of GABA synthesis, was not affected in rat cortex after parietal bone removal indicating that the operation did not activate all GABA-interneurons, but only the subpopulation which contains the peptides. Parietal bone removal strongly enhanced ipsilaterally the concentration of c-fos-mRNA in a time-dependent manner. Already after 60 min, mRNA levels of the proto-oncogene were elevated as compared to the control side. They declined thereafter to be enhanced again after 24 hours. The distribution of cells containing c-fos mRNA in rat cortex was similar to that of CCK-containing neurons.

These findings indicate that the proto-oncogene c-fos may play a mediator role in the changes in gene expression of cortical peptide-neurons.

ISCHEMIA VII

527.1

ACUTE DEFICITS IN BRAIN-CSF MAGNESIUM RESULT IN CEREBROVASOSPASM AND RUPTURE OF CEREBRAL MICROVESSELS: POSSIBLE RELATION TO STROKE. B.M. Altura, A. Gebrewold*, Q.F. Huang* and B.T. Altura*. Dept. of Physiology, State University of New York, Science Health Center, Brooklyn, N.Y. 11203.

Recent in-vivo studies from our laboratory suggest that magnesium ions (Mg^{2+}) can alter arteriolar (AR) and venular(v) tone and vascular reactivity in the peripheral microcirculation. The present studies were undertaken to determine if $[Mg^{2+}]_o$ can influence AR and V tone and reactivity in the rat cortical (pial) microcirculation in-vivo. Quantitative, high-resolution television-image intensification was utilized to perform the microcirculatory studies. Perfusion of the cerebral microcirculation with CSF containing reduced $[Mg^{2+}]_o$ (i.e., 0.3, 0.6 or 0- mM) resulted in rapid and progressively pronounced spasm of AR (15-85 μm o.d.) and V (15-120 μm o.d.) followed by irreversible rupture of V and capillaries, leading to focal hemorrhages and brain edema. Perfusion of the cerebral microcirculation with $[Mg^{2+}]_o$ > 0.8 mM was found to induce dose-dependent vasodilation (VD) of cerebral AR and V, irrespective of the route of administration, i.e., topical, IV or IA. Mg^{2+}-induced increments in diameter and blood flow occurred rapidly and in physiological concentration (μmolar range) irrespective of the salt used (i.e., $MgCl_2$, $MgSO_4$, Mg acetate, Mg aspartate HCl). Constrictor responses to phenylephrine and $BaCl_2$ were attenuated in a dose-dependent manner by all 4 Mg salts. No known pharmacologic antagonist, cyclo-oxygenase or lipoxygenase inhibitor, or opiate antagonist could modify or inhibit: 1) the cerebral vascular damage induced by low-$[Mg^{2+}]_o$ or 2) the VD induced by Mg salts. The present findings suggest that CSF and brain levels of $[Mg^{2+}]_o$ play an important role in controlling the cerebral microcirculation. Lower than normal dietary intake of Mg may be an important risk factor for stroke. (Supported in part by Research Grants from the USPHS).

527.2

SURVIVABILITY OF TURTLE AND RAT CORTICAL NEURONS UNDER ANOXIA AND ISCHEMIA IN VITRO. C.J. Doll, P.W. Hochachka*, and P.B. Reiner. University of British Columbia, Depts of Psychiatry and Zoology, Vancouver, B.C. V6T 2A9.

Turtles are extremely anoxia tolerant being able to survive for over six months while overwintering in anoxic pond bottoms. This strategy has lead to the turtle brain being used as a model for anoxia studies. Intracellular recordings from slices of rat and turtle cortex were obtained during various treatments. Turtles survived both N_2 and pharmacological anoxia ($NaCN+N_2$) for 180 min. with no noticeable effect. Rat pyramidal neurons responded with a rapid loss in membrane resistance, followed by a transient hyperpolarization, and a subsiquent depolarization to a zero membrane potential (41.3±6.5 min, N_2; 25.8±12.6 min, CN). Pharmacological ischemia (cyanide+iodoacetic acid) also caused a rapid loss in membrane resistance, transient hyperpolarization, and a rapid depolarization (3.1±.5 min, rat; 4.6±1.1, turtle). Iodoacetic acid alone had similar effects on the rat (6.5±.8 min), but the turtle had a more prolonged response (53.5±4.6 min). Ouabain caused a depolarization (8.6±1.1 min), but no initial loss in membrane resistance or a hyperpolarization. These results suggest that the turtle, which survives anoxia, is no better at surviving pharmacological ischemia than the rat. In addition, anoxia takes 13 times longer to depolarize a cell than pharmacological ischemia, and neither of these treatments is mimicked by ouabain alone.

527.3

HISTOLOGIC ANALYSIS OF FOCAL ISCHEMIC CHANGES PRODUCED BY MIDDLE CEREBRAL ARTERY OCCLUSION (MCAO). R.K.Clark, C.Fish,* E.V.Lee,* W.J.Price,* R.F.White,* G.Z.Feuerstein and F.C.Barone. SmithKline Beecham Pharmaceuticals, King of Prussia, PA 19406

MCAO in the rat produces an initial hemispheric swelling and infarction followed by a substantial decrease in hemispheric volume. Histologic analysis was used to elucidate the mechanisms of these changes. Microsurgical techniques were used to produce MCAO in spontaneously hypertensive rats. Paraffin sections of forebrains were prepared at 1, 2, 3 and 15 days post- occlusion and stained with hematoxylin and eosin, or immunohistochemically stained for GFAP. Initially (days 1 and 2), a well demarcated infarct was observed in the cortex with necrosis extending from the meningeal surface to the radiation of the corpus callosum. A zone of glial activation (with increased GFAP positivity) was present at the medial boundary with surviving brain tissue. Polymorphonuclear cells (PMN) infiltrating from the cerebral and meningeal vasculature were present at the periphery of the infarct. By day 5, the infarct was beginning to cavitate along its medial aspect. Macrophages and PMNs were more abundant, especially in discrete foci just below the meninges. By day 15, the once necrotic tissue was replaced by a fluid filled cavity. The meninges over the lesion were thickened and attached to a layer of loose connective tissue containing fibroblasts and macrophages. The medial aspect of the cyst was walled off by GFAP positive gliosis. Ischemic changes were absent in contralateral hemispheres and in sham-operated control animals. These results indicate that the initial hemispheric edema and infarction produced by MCAO are associated with necrosis, PMN infiltration and diffuse gliosis. The later loss of hemispheric volume results from phagocytosis of the necrotic tissue, concomitant with the formation of the glial scar.

526.4

RELEASE OF PLATELET-ACTIVATING FACTOR IN THE PENUMBRAL AREA OF FOCAL BRAIN INJURY IN RATS. **K.U. Frerichs, J.M. Hallenbeck", G.Z. Feuerstein' and A.-L. Sirén. Dept. of Neurology, USUHS, Bethesda, MD 20814 and 'Dept. of Pharmacol., SmithKline Beecham, King of Prussia, PA 19406**

Membrane phospholipid degradation is a rapid consequence of ischemic as well as traumatic brain injury, which could provide opportunity for production of platelet-activating factor (PAF). PAF has been suggested to be a potent mediator of injury responses in various tissues.

In the current report, the PAF content in brain microdialysis samples was assessed acutely after focal brain injury (Nd:YAG laser; Frerichs,K.U. et al.,Stroke 21:180, 1990) by a specific PAF-radioimmunoassay (DuPont). Dialysis probes (Carnegie) were inserted into the penumbral area of progressive neuronal damage surrounding the lesion core as well as into a remote control area in the parietal cortex of anesthetized rats (n=7). No detectable PAF levels were found under baseline conditions prior to injury. Acutely after injury, PAF levels in the dialysate from the injury site were 0.63±0.34ng/ml ($p<0.05$) and remained elevated throughout the observation period of 60 min (0.32±0.09ng/ml,$p<0.05$), while no PAF activity could be detected in the remote sampling site.

These data support the hypothesis that PAF, produced by the penumbral tissue, may trigger early events in the sequela of progressive neuronal death following cerebral ischemia and neurotrauma.

527.5

CHARACTERIZATION OF PHOSPHOLIPASE A_2 (PLA_2) ACTIVITY IN GERBIL BRAIN AFTER ISCHEMIA AND REPERFUSION. G.A. Rordorf*, R. A. Nemenoff*,Y. Uemura* and J. V. Bonventre. Departments of Medicine and Neurosurgery,Massachusetts Genl. Hosp., Harvard Med. Sch., Boston, MA 02114.

PLA_2 activation has been proposed as an important mechanism of ischemic brain injury based upon studies in which PLA_2 activity has been measured indirectly from tissue arachidonic acid (AA) release. We characterized PLA_2 enzymatic activity in gerbil brain after 10 min. of common carotid artery occlusion, followed by 10 min of reperfusion. Cytosolic, mitochondrial and microsomal fractions were prepared by differential centrifugation of forebrain homogenates. PLA_2 activity was assayed by release of AA from exogenous [^{14}C]AA-phosphatidylcholine. Fractions from ischemic-reperfused brains had significantly higher specific PLA_2 activities (pmol/mg protein/min):

	Cytosol	Mitochondrial	Microsomal
Control	4.3±0.4	7.7±0.6	2.2±0.2
Ischemic	6.7±0.7($p<.02$)	13.9±0.1($p<.001$)	3.0±0.2($p<.05$)

PLA_2 activity was then fractionated by gel filtration chromatography. Two forms of this enzyme were identified: cytosolic and membrane (mitochondrial, microsomal)-associated forms, migrating at approximately 60kDa and 12-14 kDa, respectively. PLA_2 activities of both forms were Ca^{2+} dependent with optimal activities at pH 8.5.

In conclusion, ischemia results in a stable activation of two distinct forms of brain PLA_2, a soluble and membrane-associated form. This stable activation of PLA_2 may play a major role in cellular injury associated with ischemia and reperfusion.

527.6

BLOOD FLOW IN THE BORDERZONE OF FOCAL CEREBRAL INFARCTS IN RATS M. Jacewicz, J. Tanabe*, X-J Wang* and W. Pulsinelli Dept. of Neurology and Neuroscience, Cornell University Medical Center, New York, NY 10021

Spontaneously hypertensive (SHR) and Fisher 344 (F344) rats had their right middle cerebral and common carotid arteries occluded under halothane anesthesia to induce focal, graded cortical ischemia (Brint et al, 1988). The rats awoke, and 24 hr later, they underwent a quantitative CBF study (^{14}C-iodoantipyrine autoradiography) and a simultaneous histologic analysis (H&E staining). CBF at the infarct border measured just under 50 ml/100g/min:

CBF (mean±SD in ml/100g/min)	SHR (N=7)	F344 (N=8)
0.5 mm within the infarct	31 ± 12*	32 ± 13*
At the infarct border	48 ± 19	44 ± 15
0.5 mm outside the infarct	74 ± 25*	61 ± 25*
Left (nonischemic) cortex	128 ± 29	---

*$p<0.05$, 2 WAY-ANOVA (vs. CBF at the infarct border)

The results are consistent with observations that the volume of cortex with CBF <50 ml/100g/min at 15 min, 1, 2, 3 and 24 hr after ischemia onset approximates the 24 hr infarct volume (Jacewicz et al, 1989, in press). The CBF threshold for ischemic damage exceeds the 10 - 17 ml/100g/min reported for larger mammalian brains (Astrup et al, 1981) and may reflect the higher baseline cerebral metabolism and CBF found in rodents (Kennedy et al, 1978).

527.7

DEXTROMETHORPHAN ALTERS CEREBRAL BLOOD FLOW AND PROTECTS AGAINST CEREBRAL INJURY AFTER FOCAL CEREBRAL ISCHEMIA. G.K. Steinberg, E.H. Lo, D.M. Kunis, and G. Grant*. Dept. of Neurosurgery, Stanford Univ. Sch. of Med., Stanford, CA 94305

Previous studies have demonstrated that dextromethorphan (DM) is neuroprotective in animal models of cerebral ischemia and it has been suggested that this effect is related to noncompetitive antagonism of neuronal NMDA receptors. We studied the effect of DM on cerebral blood flow (CBF) and cerebral injury in a rabbit model of transient, focal ischemia. Rabbits underwent two hour occlusion of the left internal carotid, middle cerebral and anterior cerebral arteries, followed by four hours of reperfusion. Ten minutes after the onset of ischemia they were treated with either i.v. DM (n=6) 20 mg/kg followed by 10 mg/kg/hr, or normal saline (NS, n=5). Regional cerebral blood flow (rCBF) was measured continuously using a laser Doppler flow meter (TSI) and in some animals with radioactive microspheres. DM attenuated the sharp, post-ischemic rise in rCBF seen with reperfusion in the ischemic core of NS controls (DM 42% pre-ischemic values; NS 108%; $p<.05$). Preliminary data indicate that DM may also prevent the delayed, post-ischemic hypoperfusion in the ischemic penumbra. DM treated animals demonstrated recovery of the somatosensory evoked potential, compared with NS controls (DM 77% pre-ischemic values; NS 20%; $p<.05$). DM's effects on CBF may contribute to its neuroprotective action or alternatively, the CBF changes may be secondary to prevention of neuronal excitotoxicity.

527.8

QUANTITATIVE COMPARISON OF MAGNETIC RESONANCE IMAGING (MRI) AND HISTOLOGIC ANALYSES OF ISCHEMIC DAMAGE FOLLOWING OCCLUSION OF THE MIDDLE CEREBRAL ARTERY(MCA). F.C.Barone, R.K.Clark, G.Z.Feuerstein, R.E.Lenkinski* and S.K.Sarkar*. SmithKline Beecham Pharmaceuticals, King of Prussia, PA 10406.

Microsurgical techniques were used to occlude the MCA in spontaneously hypertensive rats. Decreased microvascular perfusion in the ischemic cortex was verified using Laser-Doppler Flowmetry. MRI experiments were performed 2 days later using a GE 1.5T whole body imaging unit. Images of 3mm coronal sections were collected with a spin-echo pulse sequence having a TR of 2.5s and a TE of 80ms. Forebrains then were sectioned coronally (2mm), stained with 1% triphenyltetrazolium (TTC), photographed, and fixed by infiltration in 10% buffered formalin. Sections were embedded, cut (6 μm) and stained with hematoxylin and eosin (H&E). MRI images and corresponding stained sections were analyzed for the quantification of ipsilateral hemispheric swelling and infarct size using image analysis (Amersham RAS 3000). Morphological changes quantified using MRI paralleled those observed in stained sections. Ipsilateral hemispheric swelling of matched sections for MRI , TTC and H&E (range 7.1±1.3% to 9.8±3.3%) were similar and significantly correlated ($r=0.67$ to 0.75; $p<0.005$). Hemispheric infarct size of matched sections for MRI, TTC and H&E (range 38.1±3.1% to 32.9±2.5%) were similar and highly correlated ($r=0.94$ to 0.98; $p<0.001$). The identification of infarction by MRI was obvious with the MRI signal intensity in the infarcted cortex being much greater than in the normal contralateral area (increased 54.4±3.1%; $p<0.001$). Forebrain changes were absent in sham operated animals. These data indicate that quantitative MRI can non-invasively detect ischemic tissue damage with results that are well correlated to those obtained by histologic methods.

527.9

TEMPORAL ASSESSMENT OF NMR T2 RELAXATION TIMES AND DIFFUSION COEFFICIENTS OF WATER IN ISCHEMIC RAT BRAIN. R.J. Ordidge*†+, R. Knight*†+, J.A. Helpern*†+, M. Chopp*†+, L.C. Rodolosi °, and K.M.A. Welch +. + Department of Neurology, Henry Ford Hospital, Detroit, MI 48202, † Department of Physics, Oakland University, Rochester, MI 48309 and ° Parke-Davis Pharmeceutical Research, Ann Arbor, MI 48105.

The objective of this study was to investigate the utility of NMR imaging for the study of the time course of ischemic damage in brain. Middle cerebral artery occlusion (MCAO) in rats was used as a model of permanent focal ischemia. Approximately 25 animals were studied progressively from 1.5 hours to 1 week following MCAO. T2-weighted images were acquired using variable echo time two-dimensional Fourier transform (2DFT) imaging. Intravoxel incoherent motion (IVIM) imaging was used to produce diffusion-weighted images (LeBihan, D., Breton, E., Lallemand, D., et al., *Radiology* **168**:497, 1988).

The data showed a gradual increase in T2 which maximized at 24 hours post MCAO at a level of 150% that of pre-ischemic values. This was followed by a gradual decline towards normal over the following week. T2 values, however, never completely returned to normal. The contralateral side showed no variation in T2.

The diffusion-weighted images demonstrated an immediate decrease in the diffusion constant ($D\text{-}H_2O$) to 50% of normal at the earliest time point studied (1.5 hours). This was followed by a gradual increase toward a normal value at 1 week. The contralateral side showed no variation in $D\text{-}H_2O$.

We conclude that changes in $D\text{-}H_2O$ provide a measure of the early evolution of ischemia. This change in water diffusion is presumably related to changes in the structural integrity of the tissue. In addition, the variation in T2 is similar in temporal evolution to reported increases in tissue water content. Histological studies are presently in progress to establish the origin of these changes in the NMR parameters.

527.10

UNBIASED ESTIMATION OF BRAIN DAMAGE IN A RAT MODEL OF MIDDLE CEREBRAL ARTERY OCCULSION. S.M. Evans & A. Møller. Stereological Research Laboratory, Aarhus University and Neurological Research Laboratory, Hvidovre University Hospital, Copenhagen, Denmark.

Rat animal models for studying brain damage in cerebral ischaemia have been used for a number of years. However, even though these methods have been highly successful models for showing brain damage due to cerebral ischaemia, the quantitative assessment of the results has been poor. Although many attempts have been made to quantify the infarct size the methodology employed has been biased and highly inefficient. Cavalieri's principle was used to estimate the infarct volume of six rat cerebral heimispheres whose middle cerebral artery had been occluded. The method used approximately ten sections from each hemisphere and an unbiased estimate of the infarct volume could be obtained in five minutes with a coefficient of error of less than five percent.

527.11

HELIX NEURONS UNDERGO REGULATORY VOLUME DECREASE IN THE PRESENCE OF OUABAIN. F.J. Alvarez-Leefmans, S.M. Gamiño* and L. Reuss*. Dept. Physiol. & Biophys., Univ. of Texas Med. Branch, Galveston TX, U.S.A. and Dept. of Neurobiol., Instituto Mexicano de Psiquiatría, México 14370 D.F.

It is accepted that living cells prevent colloidosmotic swelling by the operation of the Na pump. Hence inhibition of the latter should lead to cell swelling and eventual lysis. Testing this hypothesis is important for understanding the mechanisms underlying neuronal volume control and because Na pump inhibition secondary to ATP depletion has been proposed as one of the factors generating cytotoxic brain edema. We have used a potentiometric technique to simultaneously measure transmembrane potential and cell water volume changes using intracellular tetramethylammonium as a volume indicator (Cotton et al, J. Gen. Physiol. 93: 649, 1989). 11 snail (Helix aspersa) neurons were exposed to 1 mM ouabain, a dose expected to inhibit the Na pump. As expected for an electrogenic pump, all cells were depolarized. 5 neurons initially swelled and down-regulated their volume rapidly. The cells started to swell within 1 min of exposure to ouabain, swelling to a maximum of 10 to 35 % above their initial volume within less than 30 sec. Then, cell volume decreased (initial rate -10 to -60 %/min). Cells not only fully recovered their volume in the presence of ouabain but actually shrank to a maximum of about 10 %. 6 neurons showed no detectable cell volume changes in the presence of ouabain.

SOMATIC AND VISCERAL AFFERENTS IV

528.1

THE EFFECTS OF PHORBOLDIBUTYRATE ON SLOWLY CONDUCTING AFFERENTS OF THE CAT'S KNEE JOINT. R.F. Schmidt, K. Schepelmann* and K. Meßlinger*. Physiologisches Institut, D-8700 Würzburg, FRG.

Inflammatory mediators, such as bradykinin and prostaglandins, which excite and sensitize nociceptive primary afferents, are proposed to bind to cellular receptors and activate intracellular second messengers. We studied the effect of the protein kinase C activating Phorboldibutyrate (PDB) on slowly conducting afferents in adult cats anesthetized with α-chloralose. Extracellular single unit recordings were made from 35 slowly conducting units, 17 belonging to group III and 18 to group IV of the medial articular nerve. In regard to their sensitivity to passive movements in the knee joint the afferents were classified as low threshold- and high threshold-units. PDB in concentrations of 10^{-6} up to 10^{-4}M was applied by an arterial bolus injection close to the joint. In 49% of the fibers studied (53% of the group III and 44% of the group IV) the discharge behavior was altered by PDB. Three distinct effects could be observed. 1. An enhancement of spontaneous activity dependent on the applied dose of PDB (12 units), 2. an enhancement of responses to passive movements in the joint (6 units), and 3. a decrease of spontaneous activity (3 units). No correlation of the effects with the mechanical threshold could be observed. From these results we conclude that in a population of slowly conducting articular afferents, nociception as well as the peripheral component of hyperalgesia are mediated partly by intracellular mechanisms which involve protein kinase C.

528.2

MULTIPLE EFFECTS OF HISTAMINE ON ELECTRICAL MEMBRANE PROPERTIES OF TRIGEMINAL ROOT GANGLION NEURONS. **B. M. Hutcheon, E. Puil, and R. M. Miura. Dept. of Pharmacology and Therapeutics, Univ. of British Columbia, Vancouver, B.C., Canada, V6T 1W5.**

Histamine was applied to in vitro slices of trigeminal root ganglion (TRG) as part of an investigation on the ability of endogenous pain producing substances to modulate sensory transmission. Slices (500 μm) were prepared from ganglia of decerebrate guinea pigs. Bath applications of histamine (1-200 μM) during intrasomatic recording produced slow, transient depolarizations in 21 neurons (1-30 mV), hyperpolarizations in 6 neurons (1-7 mV), and multiphasic responses in 6 neurons. Eight neurons did not respond despite high doses (≤ 200 μM) of histamine. The responding cells (n = 33) varied widely in their sensitivities to histamine although they individually exhibited dose-dependent responses. Input resistance increased during depolarizations (mean = +28%), and decreased during hyperpolarizations (mean = -20%). In those neurons which exhibited afterhyperpolarizations (AHPs) in the spikes evoked by current pulse injections, histamine application reduced AHP amplitude and duration. We suggest that histamine produces the depolarizing response by blocking a resting K-conductance, and the hyperpolarizing response by increasing a K-conductance. The multiphasic responses may indicate the involvement of more than one type of K-channel. The possibility is therefore raised that histamine may modulate sensory signals travelling through the TRG.

528.3

ISCHEMIC BLOCK OF LARGE FIBER FUNCTION IN REFLEX SYMPATHETIC DYSTROPHY: A PARADOX. R.H. Gracely*, S. Lynch, and G.J. Bennett. Neurobiology and Anesthesiology Br., NIDR, NIH, Bethesda, MD 20892.

Mechanical-allodynia (pain evoked by light tactile stimuli) is often observed in Reflex Sympathetic Dystrophy (RSD). Sensory assessment of 8 patients with RSD showed that 1) Just detectable sensations evoked by constant current electrical stimuli (1 sec trains, 1 msec pulses, 100 hz) applied to the allodynic area are perceived as painful, but are innocuous on the unaffected side, 2) Reaction times to painful electrical stimuli are too fast to be due to C-fiber activity, and 3) Mechanical allodynia and light touch are both absent 19-27 min after initiation of ischemic block at a time when small fiber function (warmth and cold detection) is unaffected. These three lines of evidence indicate that allodynic pain sensations are mediated by Aß low threshold mechanoreceptive (AßLTM) afferents that usually only mediate innocuous tactile sensations.

However, we have noted a paradox: in several RSD patients mechanical-allodynia is relieved by ischemic block at 2-3 min duration, well before empirical detection of any impulse blockade. Contractures and other motor abnormalities may also accompany the perceptual dysfunction of RSD. In an RSD patient with severe, chronic (24 months) contractures of all 5 toes who received an ischemic block applied to the upper thigh, we observed (and videotaped) a complete release of the contractures within 6 min. At this time there was no indication of impaired afferent or efferent transmission - sensory testing failed to show any change in detection thresholds and the patient's toes moved normally.

These results suggest that the perceptual and motor effects of these short-duration ischemic blocks are due to factors other than direct blockade of nerve transmission (e.g., hypoxia, pH change, depletion of circulating catecholamines) that may drive or enable peripheral mechanisms necessary for the expression of sensory and motor abnormalities.

528.4

NOREPINEPHRINE INDUCED SENSITIZATION OF C-FIBER CUTANEOUS NOCICEPTORS IN THE RAT. A.A. Cameron*, D.M. White and J.D. Levine* Depts. of Anatomy and Medicine., Box 0724, U.C.S.F., CA. 94143.

Norepinephrine (NE) is thought to be a mediator of sympathetically maintained pain that may occur following nerve injury. In normal animals however, NE alone has no effect on nociceptive threshold. Recent behavioural studies have demonstrated that NE-induced hyperalgesia can be produced in normal animals if NE is injected in combination with the Ca^{++} ionophore, A23187. In this electrophysiological study we examined the effects of NE alone or in combination with A23187 on mechanical thresholds of C-fiber mechano- and mechano-heat nociceptors.

Action potentials of single C-fibers were recorded from the saphenous nerve of pentobarbital anaesthetised rats (50mg/kg, ip). Receptive fields were located on the hairy skin of the hindpaw and mechanical thresholds were determined using calibrated von Frey hairs.

Intradermal injections of saline, NE or A23187 alone did not produce sensitization in 12 neurons tested. However, NE+A23187 produced a significant decrease in mechanical threshold (baseline=3.5±0.1g; post NE+A23187=2.5±0.2g; n=12; $p<0.05$) with latency to onset of 5-10 min.

These data show that local changes in Ca^{++} can induce a state in which NE elicits sensitization of nociceptors. Studies are in progress to address the cell type affected by the increase in Ca^{++} to produce hyperalgesia.

528.5

ANTIDROMIC VASODILATATION OVERRIDDEN BY SOMATOSYMPATHETIC REFLEXES IN MAN. INTRANEURAL STIMULATION AND THERMOGRAPHY. J Ochoa, D Yarnitsky, P Marchettini, R Dotson, M Cline, Good Samaritan Hosp. & Med. Ctr. and Oregon Health Sciences Univ., Portland, OR, USA

Early diffuse cooling of skin in normal hand is consistently elicited by excitation of sensory fibers in peripheral nerve trunks. In turn, delayed regional warming is also consistently recorded, provided stimulation activated nociceptor afferent fibers. We have studied, thermographically, interactions between these two physiological responses, as elicited in healthy volunteers and patients by microstimulation of median and ulnar skin-nerve fascicles. Cooling response starts rapidly after onset of stimulation, affects the whole ipsilateral hand, and less so, the contralateral. The response disappears after ipsilateral sympathectomy: it is a somatosympathetic vasoconstrictor reflex. Warming response surfaces after stimulation is discontinued, once reflex vasoconstriction relaxes. It is regionally confined to the receptive field of the stimulated nerve, persists after sympathectomy and disappears with degeneration of small caliber fibers: it is due to antidromic vasodilatation.

Once regional antidromic vasodilatation is established it persists for many minutes, to fade spontaneously thereafter. However, re-stimulation of the nerve rapidly overrides regional vasodilatation through re-engaging reflex vasoconstriction. These findings demonstrate predominance of catecholamine-mediated vasoconstrictor response over substance P-mediated vasodilator response. It is thus possible that, in nerve disease, antidromic vasodilatation triggered by ectopic discharge in nociceptor fibers may become masked by superimposed reflex sympathetic vasoconstrictor activity as a response to orthodromic, afferent, ectopic nerve impulses.

528.6

SYMPATHETIC EFFECTS ON HUMAN LOW THRESHOLD MECHANORECEPTORS. R Dotson, J Ochoa, M Cline, W Roberts, D Yarnitsky, D Simone, P Marchettini, Good Samaritan Hospital & OHSU, Portland, OR 97210

A proposed role for the sympathetic nervous system in causalgia/reflex sympathetic dystrophy (RSD) is through activation of low threshold mechanoreceptors which would in turn excite sensitized pain-signalling neurons in the spinal cord. Animal studies have shown that low threshold mechanoreceptors can be activated by direct stimulation of sympathetic efferents (Roberts 1986). We investigated the effect of sympathetic activation upon response characteristics of low threshold mechanoreceptors in normal subjects and patients with causalgia/RSD. The microneurographic (MCNG) technique was used to record receptor responses of low threshold mechanoreceptors in peripheral nerves of 8 normal subjects and 7 patients with causalgia/RSD. All units studied in patients had receptive fields within the painful/hyperalgesic areas of skin. The receptive field, mechanical threshold, and presence of resting discharge were determined for the units prior to, during, and after sympathetic activation. Sympathetic reflexes were induced by: 2-5 minutes of contralateral limb ice water immersion; startle; mental calculations; and inspiratory gasp. Changes in sympathetic efferent neural activity and effector organ response were monitored (MCNG and laser doppler capillary flowmetry).

Ten units (**5** RA, 1 SAI, and 4 SAII) in 8 normal subjects and 23 units (7 RA, 5 PC, 10 SAI, and 1 SAII) in 7 patients were studied. All units had normal stimulus-response characteristics which did not change during increased sympathetic efferent output. Resting discharges in SAII units also remained unchanged with sympathetic activation. We conclude that reflexly induced increase in sympathetic efferent neural output does not cause activation of low threshold mechanoreceptors in distal limbs in normal human subjects or in painful areas in causalgia/RSD patients.

528.7

STIMULATION OF RENAL AFFERENT NERVES INHIBITS CARDIOPULMONARY REFLEX RESPONSES IN THE CONSCIOUS RAT. S.J. Lewis, C. Barres*, H.J. Jacob* and M.J. Brody. Dept. of Pharmacology & Cardiovascular Ctr., Univ. of Iowa, Iowa City, IA 52242.

Recent electrophysiological studies have demonstrated a functional interaction between renal baroreceptor and cardiopulmonary afferents within the nucleus tractus solitarius. The present study examined the effect of renal afferent nerve stimulation (RANS) on the cardiopulmonary reflex (CPR)-mediated cardiovascular (CV) responses, produced by i.v. 5-HT, in conscious sham-operated (n=12) Sprague-Dawley rats and rats with sino-aortic denervation (SAD, 14 days post-surgery, n=8). The rats were instrumented chronically with arterial and venous catheters and an electrode on the left renal nerve. The effects of concurrent RANS (0.1 mA, 2 msec, 2.5-25 Hz for 10 sec) on the CPR-mediated CV responses were determined before and again 48 h after contralateral vagotomy. In rats with vagi intact, the CPR-mediated reductions in heart rate and blood pressure were not modified by concurrent RANS. However, in SHAM and SAD rats with contralateral vagotomy, RANS virtually abolished the CPR hypotension and bradycardia. These results indicate that afferent renal nerve activity can profoundly inhibit CPR responses by mechanisms that are independent of the baroreflex.

528.8

FUNCTIONAL EVIDENCE THAT $5HT_2$-RECEPTORS EXIST ON RAT VAGAL AFFERENT PERIKARYA. P.J. Lacolley, S.J. Lewis and M.J. Brody. Dept. of Pharmacology & Cardiovascular Ctr., Univ. of Iowa, Iowa City, IA 52242

We have shown that administration of serotonin (5-HT) into the occipital artery (OA) blood supply to the nodose ganglia (NG) of urethane-anesthetized rats produces cardiovascular effects via interaction with vagal sensory perikarya (FASEB J. 4(3):A1107, 1990). The purpose of this study was to determine the 5HT-receptor subtypes involved in the actions of 5-HT on sensory perikarya (activated by the OA route) and those on sensory terminals (iv route). The initial reductions in heart rate and arterial pressure produced by 5-HT (10-60 µg/kg)-induced activation of either vagal afferent terminals or NG cell bodies were abolished by pretreatment with the $5HT_3$-receptor antagonist ICS-205930 (100 µg/kg, iv). In contrast, the reflex hypotension and bradycardia produced by OA but not iv injections of 5-HT were markedly attenuated by the specific $5HT_2$-receptor antagonists ketanserin and xylamidine (200 µg/kg, iv) and the mixed $5HT_1/5HT_2$-receptor antagonist methysergide (500 µg/kg, iv). These results suggest that $5HT_2$-receptors, present on vagal afferent perikarya within NG, appear to mediate the component of the 5HT-induced reflex evoked by direct activation of these sensory nerve cells.

528.9

TYROSINE HYDROXYLASE EXPRESSION AND REGULATION IN NEURONS OF RODENT DORSAL ROOT GANGLIA (DRG). K.B. Seroogy, B.W. Brighton*, N.K. Mohapatra*, P.K. Lund* and E.R. Perl. Department of Physiology, University of North Carolina, Chapel Hill, NC 27599.

In contrast to the rare presence of the catecholamine cellular phenotype in rat and hamster spinal ganglia, a substantial subpopulation of small-sized neurons, 10-20% of the DRG cell population in guinea pig and mouse, exhibit immunoreactivity (I) for the catecholamine-synthesizing enzyme tyrosine hydroxylase (TH), but not for dopamine β-hydroxylase. A similar proportion expresses TH mRNA as determined by in situ hybridization with ^{35}S-labeled synthetic oligonucleotide probes complementary to regions of rat TH mRNA. Northern blot analysis of poly $(A)^+$ RNA from guinea pig DRG revealed a single TH mRNA species of expected size (1.9 kb). TH-I coexists extensively with galanin-I but rarely with substance P-I or CGRP-I. By combining TH immunocytochemistry with retrograde tracing using Fluoro-Gold, some of the TH-I perikarya of guinea pig DRG were shown to be distributed to somatic targets via the sciatic and sural nerves. Transection of the sciatic nerve in guinea pig results in a dramatic reduction in the number of DRG cells exhibiting TH-I. These data suggest that significant subpopulations of guinea pig and mouse primary sensory neurons synthesize dopamine (or L-dopa) and that the catecholamine phenotype of DRG cells is modulated by integrity of the neurons. (Supported by grants NS 08525 and NS 10321 from NINCDS.)

528.10

LABELING OF FOS PROTEIN INCREASES IN AN EXPERIMENTAL MODEL OF PERIPHERAL NEUROPATHY IN THE RAT. K.C. Kajander, S. Wakisaka*, G. Driasci* and M.J. Iadarola. Department of Oral Science, University of Minnesota, Minneapolis, MN 55455, and Neurobiology and Anesthesiology Branch, NIDR, NIH, Bethesda, MD 20892

An experimental model of a painful peripheral neuropathy has been introduced by Bennett and Xie (Pain 33:87 -107, 1988). In this model, 4 chromic gut ligatures tied loosely around the common sciatic nerve in rat produce behavioral signs of neuropathic pain. In models of tissue injury, Fos protein, the product of the *c-fos* proto-oncogene, has been used as a marker of activity in spinal cord neurons. We used immunocytochemical techniques in this study to evaluate changes in Fos labeling in the spinal cord at times between 1 and 60 days after induction of the peripheral neuropathy.

The left common sciatic nerve in each of 8 rats was ligated and a sham surgical procedure was performed on the right side. Then at selected times, rats were deeply anesthetized and perfused transcardially with 4% paraformaldehyde. After post-fixing, sections from the L-4 and L-5 spinal segments were sectioned (25 µm thick) and stained using the peroxidase-antiperoxidase method. Locations of labeled cells were reconstructed using a drawing tube at 100X.

There was an increase in nuclear labeling for Fos in the spinal cord on the side of the nerve injury at 3 and 5 days after the injury ($p<0.05$, Student's t-test). The increase in labeling occurred in both the superficial (I-II) and deep laminae (V-VII). At 14 days, the increase was still apparent but was diminished. There was no side-to-side difference in labeling at 1 or 60 days.

These data suggest that Fos labeling increases and is at its greatest soon after induction of a painful peripheral neuropathy. The increase occurs in areas of the spinal cord thought to be important in nociceptive signaling.

528.11

SEVERAL CHANNELS COMBINE TO MEDIATE THE MECHANICAL ASPECTS OF TOUCH IN HAIRY SKIN. S.J. Bolanowski, G.A. Gescheider, R.T. Verrillo and T. A.Vaughn*. Inst. Sens. Res., Syracuse Univ., Syracuse, NY 13244 and Hamilton College, Clinton, NY 13323.

We have proposed a four channel model for taction based on psychophysical experiments performed on glabrous skin and have tentatively linked afferent types to the individual channels (Bolanowski, et al, JASA, 84, 1988). It is known that hairy skin is innervated by a different compliment of receptors than is glabrous skin suggesting that psychophysical experiments on this type of skin may show an organization of channels different than found for glabrous skin. We used bursts of sinusoidal displacements (duration, 700 ms; rise-fall time, 500 ms) in the 0.4 to 500 Hz frequency range. Thresholds of human observers (n=5) were obtained. Stimuli were applied to the volar forearm through either a 2.9 or 0.008 cm^2 contactor. Skin-surface temperature was controlled at 15°, 30° and 40°C. Three channels were identified by examining threshold changes that occurred under varying conditions: 1) a low-frequency channel (0.4-2 Hz) which is temperature insensitive and affected by stimulus size; 2) a mid-frequency channel (2-70 Hz) which is sensitive to temperature changes and insensitive to stimulus size and 3) a high-frequency channel (70-500 Hz) which is slightly temperature sensitive and substantially sensitive to changes in stimulus size. These three channels for hairy skin have characteristics different than those of the analogous low-, mid-, and high-frequency channels identified in galbrous skin as would be expected from the difference in physiology and antomy of the two skin types. Whether additional channels exist for hairy skin remains to be determined.

528.12

TEMPORAL SUMMATION, THE P CHANNEL, PACINIAN CORPUSCLES AND THE NEURAL CODE FOR THRESHOLD. C.M. Checkosky and S.J. Bolanowski. Institute for Sensory Research, Syracuse University, Syracuse, NY 13244

We have suggested that the neural code used by the psychophysically defined P channel may be 2-4 neural spikes occurring per stimulus. This suggestion was based on psychophysical and physiological data obtained for short-duration stimuli but did not take into account temporal summation, a centrally mediated phenomenon known to exist for the P channel. Temporal summation has been shown to produce a decrease in threshold at a rate of -3 dB/doubling of (sinusoidal) stimulus duration, the temporal integrator having a time constant (τ) of 200 ms. We have verified this effect on 5 human observers. Additionally, Pacinian corpuscles isolated from cat mesentery were stimulated with bursts of 300 Hz displacements having durations from 10 to 1000 ms and intensities producing numbers of spikes/stimulus from 1 to 10 (short durations) or 1 to 50 (long durations). The spike train responses so obtained were passed through an energy integrator (τ =200 ms) and various threshold output values were assumed. Values of stimulus intensities and durations were selected to produce a function which coincided with the psychophysically obtained curve and the average number of spikes per stimulus required at each datum noted. The shorter duration stimuli (10-100 ms) required roughly 3-5 spikes per stimulus. Longer durations required a greater number of impulses: 250 ms required 7-10 spikes; 600 ms required 19-23. Thus it is not the number of spikes per stimulus which produces a threshold response in the system: it is probably the number of spikes per time constants of the system. Based on the above values we tentatively conclude that for the P channel, the criterion is 4-8 spikes per one time constant. Supported by NS 23933.

REGENERATION: CHAMBERS AND GRAFTS

529.1

TIME-COURSE OF REGENERATION OF ADULT DORSAL ROOT AXONS INTO TRANSPLANTS OF FETAL SPINAL CORD (FSC). Y. Itoh, M. Kowada*and A. Tessler. Dept. of Neurosurg., Akita Univ. Sch. of Med., Akita 010, Japan, Philadelphia VA Med. Ctr., and Med. Coll. of Pennsylvania, Philadelphia, PA 19129.

We have shown that cut adult rat dorsal root ganglion (DRG) axons regenerate into transplants of FSC and form synapses there. The time course over which the innervation develops is unknown, and it is also unknown whether or not the regenerated DRG axons persist. Since calcitonin gene-related peptide (CGRP) is a marker for dorsal root axons, it is also a marker for regeneration. In this study we used CGRP immunocytochemistry to label regenerated axons in FSC transplants, and quantitative stereological methods to assess the time course and persistence of axon ingrowth.

Transplants of embryonic day (E)14 spinal cord were introduced into a cavity aspirated in the lumbar enlargement of adult Sprague-Dawley rats, and the L4 or L5 dorsal root was cut and juxtaposed to the graft. Sagittal cryostat sections were prepared for CGRP immunocytochemistry after survivals of 1day to >1year. Regenerated DRG axons are present within the transplants by 4 days po and begin to form dense bundles by 1 week. The area fraction of the transplant occupied by CGRP-labeled axons increases until 3 months, and then persists unchanged for >1year. DRG axons therefore may reach the FSC transplants before a glial barrier is established, grow for several months within the transplants, and establish an apparently permanent innervation. Transplants may provide a lasting restoration of damaged neural circuits. Supported by VA Medical Research Service, NIH grant NS24707, and USAMRDC grant 51930002.

529.2

ASTROCYTES MAY CONTRIBUTE TO REGENERATION OF DORSAL ROOT (DR) AXONS INTO FETAL CNS TRANSPLANTS. K. Kikuchi, P. Levitt and A. Tessler. Philadelphia VA Medical Center and Departments of Anatomy and Neurology, Medical College of Pennsylvania, Philadelphia, PA 19129.

Transplants of fetal spinal cord support or promote regeneration of severed adult DR axons and allow synapse formation. To analyze the components of the transplants that provide this favorable environment, we studied whether or not astrocytes could support dorsal root regeneration. We used calcitonin gene-related peptide (CGRP) immunocytochemistry to identify regenerated axons because in normal dorsal horn CGRP specifically labels a population of DR axons. Astrocytes were cultured from embryonic (E) day 14 or 18 spinal cord or E18 neocortex and labeled with rhodamine microspheres to distinguish them from host astrocytes. Harvested cells (1-6 x 10^5) were transplanted as part of a Matrigel mixture or plasma clot into a cavity aspirated in the L4 segment of adult female Sprague-Dawley rats. The L4 or L5 dorsal root was cut and juxtaposed to the transplant. One month later sagittal cryostat sections were processsed for Nissl-Myelin stain and CGRP immunocytochemistry. CGRP-labeled axons regenerated into all types of fetal astrocyte grafts, but were also identified in control transplants consisting of either Matrigel or plasma clot alone. These results indicate that dorsal roots regenerate in response to various environments and suggest that one common feature is the absence of inhibitory influences known to be present in the adult mammalian CNS. Supported by VA Medical Research Service, NIH-NS24707, and USAMRDC grant 51930002.

529.3

SMALL DIAMETER CARBON FILAMENTS IMPLANTED INTO THE TRANSECTED SPINAL CORD OF THE ADULT RAT SUPPORT AXONAL GROWTH AS DEMONSTRATED BY IMMUNOFLUORESCENT TECHNIQUES USING ANTI-NEUROFILAMENT ANTIBODY. M. Dauzvardis, T. Khan, S. Sayers*, R. Hauser*, G. Gaik, and K. Burket*. Neuro-Regeneration Lab., RR&D Center, VA Hospital, Hines, IL 60141.

Carbon filaments have been shown to support the growth of embryonic spinal cord in tissue culture (Khan, T. et al. Abstr. Soc. Neurosci. 11:586, 1985) and to function as a "scaffolding" for the growth of regenerating axons in spinalized rats (Dauzvardis, M. et al. Abstr. Soc. Neurosci. 15:876, 1989). The present study was undertaken to further characterize the cellular processes observed growing into carbon filament implants positioned into the transected spinal cord of the adult rat.

Fifteen 200g Wistar rats sustained total spinal cord transection at level T8-T9 with the use of a #11 scapel blade followed by withdrawal of fine wire hook through the transection site. In ten of these animals the resulting transection gap was then filled with a 5mm long bundle of approximately 10,000 carbon filaments of 4.0μ diameter. Five rats served as surgical controls.

After a six-week survival period cryostat cut sections from both groups of animals were examined for the presence of nerve fibers using an indirect immunofluorescence technique with anti-neurofilament as primary antibody and fluorescein-conjugated IgG as secondary antibody. In surgical control animals, sections taken through the transection site were virtually devoid of fluorescent label while sections taken thru carbon filament implants demonstrated the presence of many neurofilament-positive axons.

Although these findings do not address the origin or course of the neurofilament-positive axons, they do demonstrate that small diameter carbon filaments can support and direct the regrowth of damaged spinal pathways. Supported by funds from Veterans Affairs, Rehabilitation R&D Service, Rehab. R&D Grant B423R.

529.4

SOMATOSENSORY EVOKED POTENTIALS RECORDED ACROSS CARBON FILAMENTS IMPLANTED INTO THE SITE OF A COMPLETE SPINAL CORD TRANSECTION IN THE ADULT RAT. R Hauser*, T. Khan, S. Sayers*, M. Dauzvardis, and K. Burket*. Neuro-Regeneration Lab., RR&D Center, VA Hospital, Hines, IL 60141.

Carbon filaments have been shown to support the growth of embryonic spinal cord in tissue culture (Khan, T. et al. Abstr. Soc. Neurosci. 11:586, 1985) and to function as a "scaffolding" for the growth of regenerating axons in spinalized rats (Dauzvardis, M. et al. Abstr. Soc. Neurosci. 15:876, 1989). The present study was conducted to determine whether axons observed growing across a carbon fiber implant were capable of transmitting SSEP's across a transection site.

Fifteen 200g wistar rats sustained total spinal cord transection at level T8-T9 with the use of a #11 scapel blade. In six of these animals the resulting transection gap was then filled with a 5mm long bundle of approximately 10,000 carbon filaments of 4.0 diameter. Six rats served as surgical controls. Three rats, (in which the transection gap was filled with ordinary cotton) served as substrate controls.

Noninvasive SSEP's were elicited by applying a constant current anodal stimuli to the distal tail. SSEP's were recorded at the proximal tail, lumbar-sacral spine junction, thoracic spine (T9) and cervical spine (C6-7) at one week intervals. After a 6-week survival period, a final SSEP was recorded and the animals were studied histologically. No SSEP were recorded at any time across the transection site in the regular controls and the cotton controls. Three out of six animals with carbon filament implants were found to have SSEP's, at six weeks post surgery, across the transection site that were not present in earlier SSEP recordings.

Carbon filaments by providing a favorable adhesion surface and also a possible guilding function, may prove useful in the treatment of spinal cord injury. Funding: VA, Rehab. R&D Service, Rehab. R&D Grant #B423R.

529.5

IMMUNOCYTOCHEMICAL CHARACTERIZATION OF CNS AXONAL REGENERATION THROUGH TRANSPLANTS OF SCHWANN CELL/MATRIX CABLES. R.W. Cohen*, T. Goodglick* and L.F. Kromer, Dept. of Anatomy & Cell Biology, Georgetown University, Washington, DC 20007

Prior experiments demonstrated that forebrain cholinergic axons regenerate through transplants of selectively permeable polymer tubes (ARCs) containing dissociated Schwann cells in an extracellular matrix (ECM) cable. The present study further characterizes this regeneration by identifying specific axonal and glial proteins localized on cellular processes within the regeneration cables. For these experiments Schwann cells were dissociated from monolayer cultures obtained from neonatal rat sciatic nerve and then mixed with tumor-derived ECM. This material was allowed to form a cable within the ARC before transplantation to the lesioned septo-hippocampal pathway. After survival times of 4-28 days the following observations were made on immunocytochemically stained sections: Schwann cells consistently stained intensely for the low affinity receptor for nerve growth factor (NGFR) and usually stained for glial fibrillary acidic protein (GFAP), but rarely for S-100. Glial cells from the host CNS that migrated onto the ECM cables stained for GFAP and S-100 but not NGFR. Regenerating axons (stained for GAP-43 and L1) closely associated with the Schwann cells and appeared to fasciculate near them. Much less growth occurred on the ECM cables colonized by CNS glia which lacked NGFR or L1. These results are consistent with the hypothesis that growth factors and cell adhesion molecules help mediate CNS axonal growth *in vivo*. Supported by NIH grant NS23522 and the American Paralysis Assoc.

529.6

DO AXON-FREE NERVES LEAD TO THE FORMATION OF SCHWANN CELL-CABLES WITHIN SILICONE CHAMBERS ?. A. A. Zalewski, N. A. Azzam and L. R. Williams. Lab. of Neural Control, NINDS, NIH, Bethesda, MD 20892 and CNS Diseases Research (L.R.W.), The Upjohn Co., Kalamazoo, MI 49001

After suture of proximal and distal nerve stumps into the ends of a silicone tube, a tissue cable forms through which axons regenerate. Schwann cells are critical for this regenerative success. If the distal nerve stump is omitted or skin or tendon substituted, a fibrin matrix or granulation tissue develops in the tube but axons do not regenerate into it. In this study, we sought to determine whether axons were needed to induce the formation of a Schwann cell-containing cable. The transected stumps of rat sciatic nerves were sutured into silicone chambers prefilled with dialyzed plasma, leaving a 10-mm interstump gap. In order to eliminate axons from entering the chamber, the proximal nerve was further transected, ligated and reflected, leaving a 2-mm plug of nerve in the proximal chamber. After four weeks a cable was visible that extended the length of the silicone chamber. Light microscopy revealed the presence of blood vessels and cellular elements, but the absence of myelin. Electron microscopy demonstrated the presence of Schwann cells and fibroblasts. Surprisingly, some Schwann cells were seen surrounding unmyelinated axons. These axon-associated Schwann cells developed a basement membrane whereas those free of axons did not. The blood- and perineurial-nerve barriers did not form in these cables since the barrier-tracer HRP flooded them after an IV injection. In other animals bearing a four-week cable, the reflected nerve stump was re-attached to the nerve plug in the proximal end of the chamber. Three months later, the cable and the distal nerve were filled with myelinated axons, and the perineurial, but not the blood, barrier was restored. We uncovered a source of non-myelinated axons from the distal nerve stump that needs to be eliminated to determine if axon-free tissue can form a Schwann cell-cable. Nevertheless, a cable did form that was capable of attracting axons from a re-anastomosed proximal nerve stump. A cable formed by our surgical method has the potential to be used clinically for delayed nerve repair.

529.7

CHARACTERIZATION OF RAT SPINAL CORD CHANGES FOLLOWING PHOTOCHEMICALLY-INDUCED INJURY.
A.T. Salvatierra, V.R. Holets and M.B. Bunge The Miami Project and Departments of Neurological Surgery and Cell Biology and Anatomy, University of Miami School of Medicine, Miami, FL 33136.

A model of spinal cord injury in rats was developed using a dye laser in combination with injected Rose Bengal (Prado et al., J. Neurosurg. 67:745, '87). We are characterizing this lesion prefatory to transplantation studies. A central cyst develops with regions of the lateral and ventral white matter and ventral horns remaining intact. Light and electron microscopic evaluation of lesioned spinal cords was done at 2, 5, 14, 28 and 56 d, and 4 and 6 mo post-lesioning. At 14 d, demyelination and beginning remyelination were evident outside the border of the cyst. The cystic cavity contained macrophages that were less numerous by 28 d. Also at 28 d, numerous thin myelin sheaths of both oligodendrocyte and Schwann cell origin were visible; they occurred side by side. Nonmyelinated axons were still observed. Astrocytic processes formed an intermittent border around the perimeter of the cyst. At 56 d the edema and tissue debris inside the cyst had been largely resolved, resulting in a shrunken dorsal cord and an area of ventral white matter that appeared larger than would be expected. Some Schwann cells related to axons were positioned in perivascular spaces. A surprisingly large number of axons myelinated by Schwann cells were seen in more dorsal regions, suggesting the possibility of considerable axonal regrowth with increasing time post-lesion.

(Funded by The Miami Project to Cure Paralysis and the Daniel Heumann Fund for Spinal Cord Research.)

529.8

AXON GROWTH INTO IMPLANTS OF SCHWANN CELLS PLACED IN LESIONED SPINAL CORD **C.L. Paino and M.B. Bunge The Miami Project to Cure Paralysis and Depts. of Neurol. Surg. and Cell Biology & Anatomy, University of Miami School of Medicine, Miami, FL 33136.**

Richardson and coworkers (Nature 284, '80) found that CNS axons regrew into segments of peripheral nerve implanted into the spinal cord but Kuhlengel et al. (J. Comp Neurol. 293, '90) found no axonal ingrowth into implants containing sensory neurons as well as Schwann cells (SCs). Here we test whether orphaned SCs raised in culture elicit axon ingrowth from lesioned cord tissue. SCs were allowed to ensheathe and myelinate parallel arrays of axons from dorsal root ganglion neurons in culture. Bands of longitudinally oriented SCs within basal lamina tubes were created by severing the fascicles. After undergoing Wallerian degeneration for 4 d, the collagen substratum supporting these bands of Büngner was rolled to form an implant. Rolls of collagen seeded with dissociated SCs or with no cells were also tested. Adult female rats (170-200 g) were lesioned photochemically by irradiating the spinal cord through the T9 lamina with a laser beam of 562 nm after the i.v. application of Rose Bengal. This procedure reliably causes a cystic lesion affecting the dorsal 2/3 of the spinal cord at the epicenter, and extending 4-6 mm rostrocaudally. Five or 28 days after lesioning, implants were placed into cyst cavities where they remained for 14, 28 or 90 days. Light microscopy of the spinal cords revealed good graft-host integration with a highly vascularized interface, minimal astrocytic scarring, and numerous SCs. Electron microscopy confirmed the survival of SCs and the presence of both myelinated and unmyelinated axons within the implant after 14 days. We conclude that SC implants devoid of neurons provide a more effective bridge for axonal ingrowth. (Supported by The Miami Project and the Spanish Ministry of Education.)

529.9

A COMPARISON OF DORSAL AND VENTRAL SPINAL ROOT REGENERATION M.L. McCormack, M. Goddard*, V. Guénard, and P. Aebischer. Section of Artificial Organs, Biomaterials, and Cellular Technology, Brown University, Providence, RI 02912

Semipermeable guidance channels were used to compare dorsal and ventral root regeneration across 4 mm and 10 mm gaps. Acrylic copolymer hollow fibers with a molecular weight cutoff of 50 kDa were used in a transected rat spinal root model. The 4 mm gap group was examined at 4, 10, and 24 weeks post-implantation and the 10 mm gap group was analyzed at 4 weeks post-implantation. Regeneration was assessed by quantifying the number of myelinated axons, unmyelinated axons (24 weeks only), and the endoneurial and connective tissue components of the cable cross sectional area. Both the dorsal and ventral roots regenerated across the 4 mm gap at all time periods. However, at all times the regenerated dorsal roots contained fewer myelinated axons than found in the contralateral control roots and featured an abundance of connective tissue. In contrast, by 10 weeks the regenerated ventral root contained twice the number of myelinated axons observed in the contralateral root and was composed predominantly of large, myelinated axons. DRG neuronal cell loss due to the dorsal root lesion was examined at 4 weeks post-implantation and found to be consistent with the loss documented after peripheral axotomy. Only granulation tissue devoid of myelinated axons bridged the 10 mm gap in both the dorsal and ventral roots. This study reports a difference in the regenerative capacities of dorsal and ventral roots and suggests that the environment within the semipermeable guidance channels is not sufficient for the repair of large deficit spinal root injuries. Studies involving modifications of the regenerative environment using glial cells are underway in an attempt to elucidate the factors involved in spinal root regeneration.

529.10

PERIPHERAL NERVE SEGMENTS PROVIDE A MATRIX FOR AXONAL OUTGROWTH OF AXOTOMIZED BASAL FOREBRAIN CHOLINERGIC AND ANTERIOR THALAMIC NEURONS. R.E. Clatterbuck, V.E. Koliatsos and D.L. Price. Neuropathology Laboratory, The Johns Hopkins University School of Medicine, Baltimore, MD 21205.

Fimbria-fornix resection and cingulectomy were used to remove distal axons of basal forebrain cholinergic neurons projecting to hippocampus and anterior thalamic neurons projecting to cingulate and retrosplenial cortices. Immediately following the lesion, segments of tibial nerve were apposed to the proximal stump of the fornix or cingulate bundle. Subsequently, distal ends were either externalized and placed on the parietal bone of the opposite hemisphere or connected with hippocampus or cortex. After a three-month survival, retrograde tracing from distal ends of grafts and anterograde tracing from the septum or anterior thalamus were used to study axonal regeneration within grafts. Examination of sections through basal forebrain, hippocampus, cortex, and thalamus showed that axotomized medial septal cholinergic and anterior thalamic neurons extended new axons into grafts and that these responses were associated with extended survival of axotomized neurons. To date, in survival times examined, regeneration of axons of basal forebrain and thalamic neurons do not appear to result in effective reinnervation of natural targets.

TRANSPLANTATION: EXPRESSION OF SPECIFIC NEURONAL MARKERS

530.1

IMMUNOHISTOCHEMISTRY OF HUMAN EMBRYONIC RETINA TRANSPLANTED TO ADULT RAT RETINA. [1] M. Seiler, [1] R. Aramant, [2] B. Ehinger, [2] A. Bergström* and [1] A.R. Adolph. [1] Eye Research Inst., Boston, MA 02114; [2] Dept. of Ophthalmology, Univ. of Lund, Sweden.

Human embryonic retinas (postconceptional age 3-10 wks) were grafted to the retina of immunosuppressed adult rat hosts. The donor tissue became rearranged in folded sheets or rosettes. The development of the xenografts was followed from 19 to 37 wks of total age. Immunohistochemistry was performed on Bouin's fixed paraffin sections with monoclonal antibodies for Vimentin (clone V9), S-antigen (A9-C6; gift of L.A. Donoso, Philadelphia, PA) and α-transducin (TF15; gift of B.K.-K. Fung, Los Angeles, CA). **Vimentin** stained some glial cells in the inner plexiform layer and the vitreal surface of the grafts in the earliest stages studied (19 wks). Clear staining of radial fibers and endfeet of Müller cells inside the graft was first seen at 28 wks total age. An inner limiting membrane was present inside the graft but not on the vitreal surface. At 31 and 37 wks, some horizontal cell processes and phagocytotic cells inside rosettes were stained. Both rods and cones were immunoreactive for **S-antigen**. The first staining of photoreceptors, mostly cones and some rods, with inner and occasionally outer segments was observed at 20 wks total age. At 31 wks, rods were more differentiated, showing S-antigen-positive inner and outer segments. In some darkly stained rosettes, cones were not immunoreactive. Different staining intensities of graft rosettes indicated areas of different maturation in the grafts. Similar results were obtained with the antibody for **α-transducin**. In the grafts, the first faint α-transducin immunoreactivity of inner segments and of some cell bodies was seen at 20 weeks. At 31 weeks, photoreceptor inner and outer segments were more intensively stained; cone cell bodies appeared to be mostly unstained, whereas rod cell bodies were lightly stained. This study shows that human embryonic retina transplanted to adult rat retina develops different cell types, contains proteins necessary for processing light, and can reach a degree of maturity comparable to newborn retina.

530.2

TRANSPLANTED NEURAL RETINA EXPRESSES ANTIGEN SPECIFIC FOR NORMAL DORSAL RETINA K. T. Yee and R. D. Lund Department of Neurobiology, Anatomy and Cell Science, School of Medicine, University of Pittsburgh, Pittsburgh, PA 15261

We have previously shown that embryonic mouse retinae transplanted into brains of newborn rats project to appropriate visual target regions and make functional connections. To examine the development of connectivity in more detail, we have used a mouse monoclonal antibody specific for dorsal retina (Dolce) which recognizes a 68kd-laminin receptor during the latter part of embryonic development through early postnatal life (Dräger, et al., Neurosci. Abs. 15:456, 1989). Staining with this antibody is optimal between embryonic day 13-14 but can be seen at diminished levels up to postnatal day 13. In this current study, we have examined if mouse retinae transplanted into the brains of rat hosts express this antigen.

Donor embryonic CD-1 mouse retinae were transplanted into the brains of neonatal Sprague-Dawley rats. Following survival periods of at least 5 days, animals were perfused and fixed for light microscopsy. Brains were sectioned and incubated in Dolce with 0.3% Triton. Antibody binding was visualized with an HRP conjugated secondary antibody and a diaminobenzidine nickel intensification reaction.

Our data show staining of cells in a localized region of the transplanted retinas as well as axons emerging from the transplants. This ocurrs even when the transplants are highly folded. While there can be changes in the overall anatomical conformation of the retina, staining with Dolce is still seen. This data provides further evidence that transplanted retinae develop characteristics of normal retinae.
(Supported by NIH HD 07343 and EY 05308)

530.3

VARIATIONS IN PERIVASCULAR LAMININ IMMUNOREACTIVITY DURING BRAIN ANGIOGENESIS. J.M. Krum and J.M. Rosenstein. Dept. of Anatomy, George Washington University Medical Center, Washington, D.C. 20037.

Changes in the distribution and quantity of laminin within the basement membranes (BM) of rat CNS vasculature during vascularization of fetal neocortical grafts, wound healing and perinatal development were investigated using two immunocytochemical techniques. In paraffin sections, which were routinely enzyme pre-treated, all vessels reacted with antilaminin; those at graft or wound sites in adult brains were the most intensely stained. In immunoprocessed 40μ vibratome sections of normal adult brains, only the meninges and vasculature of circumventricular organs were stained; CNS parenchymal vessels reacted only if the sections were enzymatically pre-treated. In contrast, the nascent vasculature in developing brain and the regenerating vessels at transplant and wound sites were strongly immunoreactive without enzymatic pretreatment. Electron microscopic examination revealed that laminin immunoreactivity decreased after perivascular astroglial contact occurred. The existence of a variant form of laminin, differences in vascular BM structure or differences in neuropil density between developing or injured and intact adult brains might account for these observations. (NIH-NS-17468)

530.4

CALBINDIN D-28K IN EMBRYONIC BASAL FOREBRAIN GRAFTS TO ADULT RATS. S.Shoham and E.Wertman*, Dept. Neurogeriatric Research, Sara Herzog Hospital, Jerusalem 91001, Israel.

In Alzheimer's disease (AD) there is substantial reduction in the number and size of cells containing the calcium binding protein calbindin D-28K (CB) in cortex and in nucleus basalis of Meynert (nbM) (Ichimiya Y. et al., Brain Res., 475:156, 1988). To explore relation of CB cells to the cholinergic nbM-cortical system which also degenerates in AD, adult rats received ibotenate damage to nbM and then grafts of embryonic rat E16 basal forebrain (the primordial nbM), to cortex (methods of Fine et al., Neuroscience 16:769, 1985). After 3-8 months CB neurons were identified immunohistochemically and cholinergic regions by acetylcholinesterase (AChE) histochemistry (7 rats, 14 grafts) in 25um thick sections. There were 37±5 CB cells/mm^2 in graft AChE patches, significantly more (paired t-tests) compared to 17±4 CB cells/mm^2 in AChE-negative graft regions, and to 4±1 CB cells/mm^2 in the areas of the host deep cortex and corpus callosum where grafts were placed. Some CB-neurons in AChE patches extended axons into the host cortex. This is preliminary evidence that CB-neurons of nbM are part of the cholinergic nbM-cortical system and that CB is expressed in nbM neurons even without their normal afferent inputs from amygdala, striatum and other regions.

530.5

DEVELOPMENT OF SYNAPSIN I AND SYNAPSIN II IN INTRAOCULAR HIPPOCAMPAL TRANSPLANTS. A-Ch Granholm[1], E. M. Dudek[2], H. Bergman*[1], and M. Browning[2]. [1]Department of Cell Biology, Univ. of Linköping Fac. Health Sci, Linköping, Sweden, and [2]Department of Pharmacology, Univ. of Colorado Health Sci. Ctr., Denver, Colorado. Synapsin I and II are synaptic vesicle-associated neuronal phosphoproteins that are thought to play a role in the regulation of neurotransmitter release. The levels of these proteins are low in cortical regions of newborn rats but exhibit a dramatic increase postnatally, parallel to synaptogenesis. The aims of the present study were to evaluate whether hippocampal grafts would follow a normal time course of synapsin development. Hippocampal tissue was dissected from rat fetuses of embryonic day 18 and transplanted to the anterior chamber of the eye of adult hosts. Grafts and *in situ* tissue was homogenized in 1% SDS-solution or fixed and sectioned on a cryostat for immunohistochemistry. The levels of synapsin I and II were assayed by immuno-blot methods. The following time periods were used: fetal day 18, birth, 1, 2, 4 and 8 weeks postnatally. The transplants developed levels of synapsin close to the *in situ* levels, with no obvious delay due to transplantation. The ratio of synapsin I and II reflected a CNS rather than a PNS ratio. The histological analysis showed a similar development, with accumulations of synapsin-positive profiles adjacent to the pyramidal cell layer. Synapsin levels in aged transplants are under investigation. In conclusion, these studies demonstrate biochemical evidence of a close to normal development of isolated hippocampal formation transplants *in oculo*. Supported by the Swedish Medical Research Council grant 8650 and NIH grants AG04418, DK40483 and NS26377.

530.6

TRANSPLANTS OF MIGRATORY GNRH CELLS INTO THE BRAIN ARE CAPABLE OF INDUCING GONADAL RECOVERY IN HYPOGONADAL (HPG) MICE. I. Livne, M. J. Gibson and A. J. Silverman. Dept. of Medicine, Mount Sinai Sch. of Med., New York, N. Y., 10029 & Dept. of Anat. Cell Biol., Columbia Univ., New York, N. Y. 10032.

GnRH neurons are derived from the olfactory placode and migrate into the CNS during embryogenesis. In this study we examined the capability of transplants containing this migratory population of GnRH cells to establish functional connections with the median eminence (ME), and hence to induce gonadal recovery in mutant hpg mice. Nasal area tissue from normal mouse embryos at E12 or E13 was grafted bilaterally into the anterior hypothalamus or preoptic area of adult hpg males (n=9). Following survival of 2 to 8 weeks the animals were perfused, their testes weight recorded, and the brains processed for GnRH immunocytochemistry. In 4 animals the grafts proliferated across the midline, with some tissue entering the third ventricle. Of these, L28 showed gonadal recovery (testes weight of 23.6 mg, mean weight of untreated hpg testes: 7.5 mg). This recovery corresponded to the presence of a small number of GnRH cells in the graft and a sparse innervation of the ME. L20 had a much more robust ME innervation but due to early sacrifice (20d) gonadal recovery was not yet evident. In those animals where the graft remained wholly within the parenchyma, testicular recovery did not occur. However interesting observations were made. In N31 GnRH cells appeared to migrate out of the graft into the host and these cells elaborated long bifurcating axons some of which coursed medially in the direction of the third ventricle. In L30 migration of cells into the host was less clear cut, but again GnRH axons grew toward midline and then headed ventrally to the ME. These observations suggest that migratory GnRH neurons when grafted into the brain of adult hpg mice retain some migratory potential and can elaborate extensive axonal projections, which upon innervation of the ME can induce gonadal recovery. NS 20335.

530.7

PINEAL GLAND TRANSPLANTS INTO THE CEREBRAL HEMISPHERE OF NEWBORN RATS REVERSE THE EFFECTS OF PINEALECTOMY ON SERUM MELATONIN LEVELS. J.A. McNulty, R. Swenson, R.J. Handa, T. Hogan*, P.L. Shaw*, B.S. Klausen*, L.Kus, A.J. Castro. Department of Cell Biology, Neurobiology and Anatomy., Loyola University, Maywood, IL 60153

The functional activity of neonatal pineal glands grafted into the cerebral hemisphere of littermate rats (0-1 day old, Long Evans, black-hooded) was tested in 4 groups: unoperated controls (C; n=7); pinealectomized rats (PX; n=6); pineal transplants (PT; n=6); and pineal transplants that had been pinealectomized (PT+PX; n=7). Using hypothermic anesthesia, pineal glands were placed into small cortical lesion cavities made rostral to bregma immediately before grafting. At 4-5 months, rats were chronically cannulated and serum melatonin levels measured by RIA (CIDTech) during the light and dark period of the 24-h L:D cycle (10 h L: 14 h D). A significant nocturnal decline in melatonin was observed between C vs PX ($p<0.05$), but not between C vs PT and C vs PT+PX. These findings indicate that pineal transplants were functionally capable of restoring nocturnal levels of serum melatonin. Elevated daytime melatonin levels in the PT+PX group suggests that some transplants may have been free-running.
(Supported by NSF #BNS-88-1726, NIH #NS-13230).

530.8

IMPLANTATION OF FETAL BASAL FOREBRAIN CELLS INTO CAUDATE OR NUCLEUS BASALIS AFTER LESION OF THE RAT NUCLEUS BASALIS: A NEUROANATOMICAL STUDY. L.Lescaudron, R.L.Sutton and D.G.Stein. Brain Research Lab.,Rutgers Univ.,Newark,NJ 07102.

The effects of delay (8-17 d) between lesion and transplant (TP) and delay (1-4.5 h) between harvest and TP of fetal (E15-E17) cholinergic cells into the lesion site or the caudate n. (CN) were examined in 16 rats with ibotenic acid lesion of the left n. basalis magnocellularis (NBM). Brains were processed for cresyl violet, ChAT- and GFAP-ICC, and for AChE and cytochrome oxidase (CO) 2 months after TP. Survival was always poor in the CN, with small, immature TP-cells, extensive gliosis, and low levels of CO activity. In the NBM, the best TP survival was observed for fetal cells implanted less than 2h after harvesting and 14-17d postlesion. In these cases large, healthy TP-neurons displayed robust CO activity. Also an intense cholinergic innervation was observed within these TPs, with TP-cholinergic neurons sending processes throughout the TP. A small astrocytic reaction was noticed within TPs and at the host-TP interface. In about 1/3 of the TPs, a Status Marmoratus-like profile was noted. The effects of TPs on the host tissue will also be presented.
Supported by FIDIA and 9R01 NS 25685-05

530.9

GAP-43 IMMUNOREACTIVITY DURING THE DEVELOPMENT OF TRANSPLANTED FETAL MESENCEPHALIC NEURONS. G.H.Clayton, T.J.Mahalik, T.E.Finger. *Dept. Cell. & Struct. Biol.; UCHSC; Denver, CO 80262*

GAP-43 is a neuron-specific phosphoprotein which is differentially regulated during normal development. High levels of this protein are correlated with axonal elongation in-vitro, regeneration in-vivo, and highly plastic neural structures such as the hippocampus and olfactory bulb. Work in our laboratory shows that in the developing rat nigrostriatal pathway GAP-43 immunoreactivity is highest during the 6 day period E15 until birth at which time it declines rapidly to adult levels. This time course corresponds with the period of axonal extension for the majority of the substantia nigra dopaminergic neurons. In the present study we use an antibody against GAP-43 to characterize the time course of development of transplanted fetal mesencephalic neurons. For these experiments 6OHDA lesions of the nigrostriatal tract were made in Sprague Dawley rats. Adequacy of the lesion was determined by apomorphine-induced rotation prior to the transplantation of pieces of ventral mesencephalon (VM) obtained from E15 fetuses. Immunocytochemistry revealed high levels of GAP-43 at 5, 11 and 15 days post transplant but lower levels at 3 weeks. By 13 weeks the immunoreactivity present within the transplant tissue is equivalent to normal background levels within the host neuropil. Thus, the presence of high levels of GAP-43 immunoreactivity is prolonged compared to the developing VM in situ. This suggests that axon elongation occurs over a longer period in VM grafts than in situ. Such retarded graft development could be the result of a number of different phenomena such as: surviving cells whose processes were sheared off during surgery may reinitiate axonal elongation; the altered environment of the host may not contain trophic factors in sufficient quantities to support rapid growth; or a lack of normal targets for grafted neurons may extend their growth period in a search for alternatives.

531.1

NUCLEAR MAGNETIC RESONANCE (NMR) IMAGING OF FETAL BRAIN GRAFTS N. HAWRYLAK, P. GHOSH, J. BROADUS, W.T. GREENOUGH and P.C. LAUTERBUR Beckman Inst., Neurosci. Prog., Dept. Psych., & Biomed. Magnetic Resonance Lab., Coll.of Med., Univ. of Illinois, Urbana 61801

Progress in neural transplantation would be aided by improved monitoring of the fate of donor tissue. We are developing techniques for in vivo nuclear magnetic resonance (NMR) imaging of grafted neural tissue.

Fetal rat (E17-E18) posterior neocortex was employed in grafting studies by two methods: (a) **Neonatal** (P0-P1) host rats had fetal tissue fragments grafted into posterior cortex. (b) **Adult** (P150) host rats received grafts as dissociated cell suspensions, labeled with iron oxide particles using reconstituted Sendai viral envelopes. NMR imaging of the adult and neonatal hosts was performed on post-transplantation days 10 and 120 respectively. Histochemistry (Prussian blue test for ferric iron) and immunohistochemistry (GFAP) of host brains were performed on 30μ thick adjacent sections.

In the NMR images of intact perfusion-fixed neonatal host brains (excitation slice thickness=700μ; pixel= 60μ x 60μ; TE=55ms; TR=4s), the tissue fragment grafts appear equivalent to the surrounding cortical structures but bright with respect to the immediately adjacent white matter.

Images (excitation slice thickness=700μ; pixel=150μ x 150μ; TE=30ms; TR=4s) of the brain of adult hosts, both in vivo and after fixation, showed dark regions . Light microscropy of these regions demonstrated iron oxide positive cells of probable donor origin. The iron oxide appeared as a dark blue precipitate within soma and proximal processes, suggesting good short term survival of the donor cells. The exact location of the exogenous iron and the possible gliotic response within the host tissue are currently being investigated. Supported by ONR N00014-89-J-1556

531.2

TRANSMISSION ELECTRON MICROSCOPY OF WHOLE FETAL HIPPOCAMPAL TRANSPLANTS INTO LESION CAVITIES. R. H. Baisden and M. L. Woodruff. Dept. of Anatomy, East Tenn. St. Univ., J. H. Quillen Coll. of Med., Johnson City, TN 37614

Implants of fetal hippocampus were placed into aspiration cavities in adult rat brains. After 45 days the animals were killed and perfused with Karnovsky's fixative and processed for electron microscopy. Elements typical of the matured hippocampus were observed. These included small and large neurons, glial cells, and a typical neuropil including axon bundles, dendritic fields, and regions of high synaptic density. A group of small multipolar cells were found. These cells showed a very pale cytoplasm with prominent mitochondria, lysosomal bodies and bundles of filaments scattered throughout the cytoplasm. Many of these cells appeared multinucleated. Macrophages were also found within the transplants. The surfaces of the transplants were covered with ciliated and nonciliated ependyma or were bounded by an external membrane. In many areas bundles of processes exited the neuropil onto the surface of the transplant. Some of these processes contained vesicles typical of axons. Supraependymal cells could be found. Some of these had characteristics of neurons; some of glia; and some appeared to be macrophages. (Supported by a grant from the NIH (ES04070-04) to MLW.)

531.3

ULTRASTRUCTURAL CHARACTERISTICS OF FETAL SUPRACHIASMATIC NUCLEUS TRANSPLANTS IN LATERAL AND THIRD VENTRICLES AND IN CULTURE. U. Vaidya and R. Y. Moore. Dept. of Neurology , SUNY, Stony Brook N.Y. 11794

Fetal suprachiasmatic nucleus (SCN) transplants have been shown to restore circadian rhythmicity in SCN lesioned hosts if the transplants are located in the thrid, but not the lateral ventricle. In the present study, SCN transplants in each location and in culture were examined to detect any site specific differences in development. Female Sprague-Dawley host rats received 2 to 3-1 mm^3 pieces of SCN from E16 donor fetuses injected unilaterally into the lateral ventricle. Explants of 200 u slices of SCN from the same set of donors were placed in culture. Hosts with transplants were allowed to survive for 30 days, while cultrues were examined at 2 or 4 weeks after explanting. Sections of brains from host animals were processed for VIP immunocytochemistry and electron microscopy. Cultures were fixed *in situ* and processed similarly. In all host animals, transplants developed in the lateral ventricle and at least one piece of transplanted tissue developed in the third ventricle. Infrequent discontinuities in the ependymal lining allowed direct contact between the host and transplant and the passage of axons. The degree of appostion of the transplant to the host brain was greater in the third ventricle. Transplants in both locations and explants in culture had VIP positive neurons, and in the neuropil, myelinated and unmyelinated axons, and synapses. Blood vessels were also seen in transplants. The organization of neuropil in cultured explants was less complex than transplants in hosts. The results suggest that SCN transplants into the lateral and third ventrilcle have a similar organization and development. The greater degree of host-transplant apposition in the third ventricle was the only notable site-related difference.

531.4

LONG TERM IDENTIFICATION OF TRANSPLANTED RODS. P. Gouras, J. Du*, R. Kwun*, R. Lopez*, H. Kjeldbye*. Columbia University Department of Ophthalmology, 630 W. 168 St. New York 10032

Rat rods in mitosis (at least 50%) can be radiolabeled by administering ^{3}H-thymidine subcutaneously to newborn rats. At 1-2 months of age these rods are isolated enzymatically from the retina, mixed with dissociated retinal pigmented epithelial cells from non-labeled donors and transplanted to the subretinal space of 4-5 month old congenic albinotic dystrophic RCS rats. At this time the dystrophic rat has virtually no rods. The transplant site is identifiable by the speckling of pigmented epithelium in the albinotic retina observable both ophthalmoscopically and histologically. Examination of the host retinas after transplantation reveals the unequivocal nuclear stain of tritiated thymidine in these transplanted rods at three months (longest time) after transplantation. Electronmicroscopy reveals that these rods can have spherules and abortive outer segments, the former containing synaptic vesicles, ribbons and forming synaptic complexes. The postsynaptic cells are presumably host because they are neither rods nor pigmented epithelial cells.

531.5

ADRENAL MEDULLA SURVIVES FOR LONG TIME PERIODS AND PREVENTS DEGENERATION OF THE SUBSTANTIA NIGRA WHEN CO-GRAFTED IN THE PNS. L.C. Doering and M.A. Tokiwa*. Department of Biomedical Sciences (Anatomy), McMaster University, Hamilton, Ontario, CANADA L8N 3Z5.

Grafts of fetal substantia nigra (SN) undergo a series of degenerative changes when transplanted into the peripheral nervous system (PNS). This study was performed to determine if the adrenal medulla (AM) could prevent the death of SN neurons when combined in the PNS.

The sciatic nerves of Lewis rats (180-200g) received single embryonic day 15 SN grafts or co-grafts of SN plus young adult AM. Transplants were examined after one year by immunohistochemistry with antibodies against tyrosine hydroxylase (TH), the rat nerve growth factor receptor (192-IgG), phosphorylated (RT97) and non-phosphorylated (SMI-32) neurofilament proteins.

Single nigral grafts were shrunken, contained only a few TH positive cells and displayed abnormal patterns of RT97 and SMI-32 immunoreactivity. In contrast, when combined with the AM the loss of TH neurons was prevented and the SN did not show the aberrant cytoskeletal changes. Furthermore, all AM grafts were viable after one year in the peripheral nerves and consisted of hundreds of chromaffin cells identified by expression of the TH and 192-IgG epitopes.

These experiments illustrate that the PNS can support grafts of AM for up to one year and the AM can offset the degeneration of SN neurons when co-grafted under the present experimental conditions. (Supported by The Parkinson Foundation of Canada)

531.6

INFLUENCE OF AFFERENT INPUT ON SURVIVAL OF FETAL NEURAL TRANSPLANTS INTO A HOST TARGET. J.H. McLean and A.H. Darby*. Div. of Basic Medical Sciences, Memorial Univ. of Newfoundland, St. John's, Nfld., Canada, A1B 3V6.

In Alzheimer's disease, several regions of the brain degenerate, including cortical structures such as the olfactory bulb and several afferent inputs to cortices. One means of providing replenishment of depleted pathways in the brain is to provide the brain with trophic and/or neural transplants. In these experiments, we have transplanted fetal rat (E15-E17) cholinergic and serotonergic neurons from the diagonal band and raphe nuclei, respectively, into olfactory bulbs of adult rats that have been previously depleted of cholinergic (ibotenic acid into diagonal band) or serotonergic (5,7-dHT into anterior olfactory nucleus) afferent inputs. The contralateral olfactory bulb and non-depleted animals served as control for influence of afferent inputs/trophic substances on transplant survival. Following survival of 4 to 8 weeks, the animals were sacrificed by perfusion and the olfactory bulbs were cut frozen at 30 μm. The sections were analyzed by immunocytochemistry and AchE histochemistry to determine the location and degree of integration of transplanted cells into the host brain. Cholinergic and serotonergic neurons generally exhibited good survival and integration into the host olfactory bulb. Transplanted cholinergic neurons on the side ipsilateral to cholinergic depletion and serotonergic neurons on the side ipsilateral to serotonin depletion survived better than transplanted cells on the contralateral side. In addition, transplanted neurons appeared to survive less well in animals that had not previously been depleted of afferent input to the bulb. These results suggest that trophic substance released in the target as a result of afferent depletion increased the survival of transplanted cells and the trophic effect may not be limited to the side of the brain that was previously depleted of transmitter. This work was supported by the American Health Assistance Foundation and MRC of Canada.

531.7

AUTORADIOGRAPHIC STUDY OF FETAL STRIATAL GRAFTS PLACED IN HOST STRIATUM PULSE-LABELED WITH [^{3}H]-THYMIDINE. F.-C. Liu, A.M. Graybiel and S.B. Dunnett, Dept. Brain and Cognitive Sci., MIT, Cambridge, MA, USA, and Dept. Exp. Psychology, Univ. of Cambridge, Cambridge, UK.

A fundamental issue in assessing fetal striatal grafts is whether the resulting graft, which is usually surrounded by a ring of gliotic tissue and fibers, contains cells that migrated from the host striatum. In the present study, we pulse-labeled host striatal cells with [^{3}H]-thymidine to test for possible spatial interactions between host and donor cells in the zones of intrastriatal grafting. Three groups of host rats were pulse-labeled with [^{3}H]-thymidine at embryonic days E12-E15 or E12 and E15-E18 or E12 and E16-E19, and were allowed to reach maturity. One week prior to grafting, ibotenic acid-induced lesions were made unilaterally in the host striatum. At grafting, dissociated cells from E15 rat striatal primordia were injected bilaterally into the host caudoputamen. Tissue was processed for autoradiography 8-9 months postgrafting. Despite the presence of many intensely-labeled cells in the host striatum of rats in all three groups, very few intensely-labeled cells were found in the cores of grafts either in the ibotenate-treated or in the intact host striatum. A few weakly-labeled small cells appeared in the graft cores, and a few strongly- or weakly-labeled cells appeared at the margins of the graft zones. Some perivascular cells associated with blood vessels in the grafts were also weakly labeled, but the gliotic tissue surrounding the graft zones was not labeled. These results suggest that few striatal cells migrate into the cores of graft zones, or that, if they do, such cells do not survive. At most, a few surviving host striatal cells have limited spatial interactions with donor cells at the margins of the grafts, both in the damaged and intact host striatal environment. These observations, combined with our previous finding that [^{3}H]-thymidine-labeled cells derived from E15 striatal primordia do not appear in the host striatum, indicate that no extensive mutual migration between striatal donor neurons and host neurons occurs in the zones of grafting. Supported by NSF BNS 8720475 and NATO grant RG.85/0180.

531.8

A PARTIALLY PURIFIED MUSCLE FACTOR INDUCES CATECHOLAMINE DIFFERENTIATION IN THE LESIONED AND GRAFTED RODENT BRAIN L. Iacovitti and J. E. Springer, Institute for Neuroscience, Dept. of Neurology, Hahnemann University Philadelphia, Pa. 19102

Our previous studies have demonstrated that a partially purified muscle factor called MDF induces novel expression of the catecholamine (CA) enzyme tyrosine hydroxylase (TH) in cultures of embryonic caudate nucleus (CN) and greatly amplifies TH expression in the CA neurons of cultured substantia nigra. In the present study, we examined whether MDF retains biological activity in vivo by testing its ability to 1) induce TH expression in embryonic CN neurons transplanted into the lesioned adult brain and 2) affect TH expression in CA fibers originating from the host substantia nigra. The CN of adult Sprague-Dawley rats was partially denervated after unilateral injections of the 6-hydroxydopamine into the substantia nigra. Lesions were verified by monitoring rotational behavior after amphetamine challenge (5mg/kg). Ten-14 days after the lesion, a suspension of $3x10^5$ CN cells from embryonic rats (E15) was transplanted into the denervated adult CN. Rats were simultaneously implanted with an indwelling canula placed immmediately adjacent to the transplant and connected to a mini-osmotic pump containing either partially purified MDF or PBS (control). Test solutions were delivered at a rate of 1ul/hr for a total of 72-168 hrs. To examine the effects of MDF on local CA fibers in the CN, some rats were infused with MDF but did not receive CN transplants. Brains were then processed for the immunocytochemical localization of TH. In MDF-treated rats, those transplanted CN neurons that became well integrated into the brain parenchyma were TH immunoreactive, and extended long neuritic processes into the host CN. In MDF-treated rats which did not receive a CN transplant, an extensive network of host TH fibers with swollen endings was observed adjacent to the infusion site. These findings suggest that MDF retains its biological activity in the adult brain, capable of both inducing CA differentiation in transplanted embryonic CN neurons and increasing TH expression in local CA fibers from the host adult brain. Supported by NIH NS 24204-01A2.

531.9

FETAL LIVER AND MUSCLE GRAFTS DO NOT PROTECT AGAINST QUINOLINIC ACID STRIATAL LESIONS. M. Levivier[1], A.A. Hurrell*[1], S.H. Pearlman[2], D.M. Gash[2] and J. Brotchi*[1]. Dept. Neurosurgery, Erasme Hospital, Universite Libre de Bruxelles, Brussels, Belgium[1] and Dept. Neurobiology and Anatomy, University of Rochester, Rochester, NY 14642[2].

We have recently reported that fetal striatal grafts in the adult rat striatum protect the host against the damages produced by a subsequent local injection of quinolinic acid (QA) (Levivier, M. et. al., *Soc. Neurosci. Abstr.*, 15:1355, 1989). One of the mechanisms of this protective effect could be a specific trophic effect generated by the fetal striatum.

In order to confirm the tissue specificity of this phenomenon, our group has begun to study grafts of various types of tissue. In the present experiment, we tested the effect of transplants of embryonic non-neuronal tissues known to have trophic activity. Using the same experimental paradigm, i.e. two unilateral intrastriatal grafts, followed 10 days later by an ipsilateral QA injection, we compared transplants (n=10/group) of fetal striatum, fetal liver, fetal muscle, sham transplants and non-transplanted animals. The data show that transplants of fetal muscle and liver provide a variable but not statistically significant protection against QA. However, significant protection was again seen with fetal striatal grafts, indicating some specific effect of this tissue upon the host brain. Studies are continuing to elucidate the mechanisms underlying the fetal striatal graft-induced protective effect. Supported by NIH NS 15109.

531.10

CHOLINERGIC GRAFTS INNERVATE THE RETROSPLENIAL CORTEX IN A DIFFUSE PATTERN. S.R.Keim, S.L.Gage and W.C.Low.,Prog. in Med. Neurobiol. and Dept. of Physiol. and Biophys., Indiana Univ. Sch. of Med., Indpls., IN 46202.

Recent studies have shown that the retrosplenial cortex (RSC) plays a role in spatial memory function and habituation. In the hippocampus (HC) the cholinergic system has been shown to be closely associated with similar functions of learning and memory. It is therefore notable that like the HC the RSC receives its cholinergic input from the medial septal nucleus (MSN) and the diagonal band of Broca (DBB), unlike lateral cortex which receives cholinergic input from the nucleus basalis. With the present study we begin to assess the role of the cholinergic projection to the RSC by developing a model of cholinergic transplantation into the denervated RSC. The RSC of 8 animals was cholinergically denervated by performing combined cortical/fornix aspiration lesions (Gage, et al., Neurosci.Abs., 1990). Four animals then received bilateral 0.5 ul injections of a suspension of fetal MSN/DBB cells (16 days gestational age) in Hank's balanced salt solution (HBSS), using a 1 ul Hamilton syringe (-5.8 mm AP; 0.7 mm ML; -2.2 to - 1.0 mm DV). Two of the other lesioned animals received similar injections of HBSS alone, while the final 2 lesioned animals received no injections. Two additional animals served as non-lesioned controls. Survival time was 4 weeks, at which time all 10 animals were perfused and placed in 30% sucrose in 0.1 M phosphate buffer for 3-5 days prior to sectioning at 50 um with a freezing microtome. Alternate sections were processed for ACHE histochemistry or cresyl violet (CV) histology. The CV stain in non-lesioned controls displayed the normal cortical cell layers I-VI of the RSC. In addition, the normal controls showed the ACHE staining pattern typical to the RSC, including an increased density of staining in cell layers I and IV, with a more diffuse staining pattern in layers II, III, V and VI. Lesioned animals without transplant (both with and without injection) all displayed normal cellular histology per CV, but showed a complete and consistant lack of ACHE staining in all cell layers of the RSC. Lesioned animal with transplants displayed normal cellular histology per CV, with the addition of clusters of putativly transplanted cells near the injection site. These animals also displayed the presence of ACHE staining from 1.5 to 2.0 mm surrounding the injection site, in a predominantly non-laminar pattern. While transplant survival and diffuse ACHE staining of all cell layers was present in all 4 transplant animals, only one RSC transplant displayed an area of increased staining density in cell layers I and IV limited to approximately 600 um surrounding the injection site. This study indicates that while it is possible for cholinergic transplants into the denervated RSC to survive 4 weeks and extend fibers, the normal ACHE lamination was not regained.

531.11

AN ORGANOTYPIC ROLLER TUBE COCULTURE OF NEONATAL RAT STRIATUM AND ADRENAL MEDULLA. C.Spenger[1]*H.-R. Lüscher[2] and H.J. Reulen[1]* Dept. of Neurosurgery, Inselspital[1], CH-3010 Bern; Dept. of Physiology[2], University of Bern, CH-3012 Bern, Switzerland.

Some beneficial effects of autologous adrenal medulla implants in patients with Parkinson's disease have been reported. Adrenal medullary implants in unilaterally 6-OH-dopamine-lesioned rats have repeatedly been shown to reduce apomorphine-induced turning behavior of these animals. The physiological mechanisms underlying these behavioral changes are not understood. In order to study possible cellular interactions between chromaffin and striatal cells an *in vitro* model was developed.

The caudate-putamen (CP) and adrenal glands were dissected out of newborn rats. By means of a tissue chopper the CP was cut into 350 µm thick coronal slices. With a cut off and sharpened hypodermic needle pieces of 2.0 mm diameter were punched out of these slices. The adrenal gland was cut into 150 µm thick sections. Sections with a large portion of medulla were selected and cut in half. A piece of CP together with a piece of adrenal gland was mounted on a cover slip in a drop of chicken plasma, coagulated with thrombin. The cover slips were transferred to a tube and fed with 1.1 ml of medium containing 5 ng/ml NGF. The cultures were incubated in a roller drum. Details of the culture technique used are described by Braschler et al. (*J. Neurosci. Meth.* 29: 1989).

Within two weeks *in vitro* the explanted tissues flattened nearly into a monolayer. Numerous large multipolar, cholinergic neurons could be seen in the CP explant. A large number of neurons did not stain for AChE and probably represented the GABAergic neurons of the CP. On the surfaces of these cells, synapse-like cholinergic nerve terminals were seen. Neurites from the cholinergic neurons had grown into the adrenal coexplant where they formed a fine varicosity-rich plexus among the chromaffin cells, to which they formed close contacts. The adrenal medullary cells stained positive for AChE and their somata gave rise to several neurites. The large CP cells were easily accessible for microelectrode exploration, and action potentials could be evoked by injection of small depolarizing current pulses.

These preliminary results demonstrate that this *in vitro* model can be used for investigating cellular interactions between striatal and adrenal medullary cells.

531.12

VASCULARIZATION AND MICROVASCULAR PERMEABILITY IN SOLID VS. CELL SUSPENSION EMBRYONIC NEURAL GRAFTS. K. E. Leigh, K. Elisevich and K. A. Rogers*, Dept. of Anatomy, The University of Western Ontario, London, ON N6A 5C1

Postmortem study of transplanted adrenal medullary tissue in human striatum has shown poor survival raising questions regarding the adequacy of vascularization. With the advent of human fetal neural transplantation, similar concerns are raised. Vascularization (VAS) and microvascular permeability (MVP) of solid and cell suspension preparations of rat embryonic mesencephalic tissue (E 13-15) were studied after transplantation in adult rat striatum deafferented by injection of 6-hydroxydopamine into the median forebrain bundle. Both VAS and MVP were assessed following intravascular infusion of horseradish peroxidase (HRP; M.W. 40 000) after 7, 14 and 30 days survival. Vascularity and HRP leakage from grafts were studied using tetramethylbenzidine (TMB) as the chromogen. Tyrosine hydroxylase (TH) immunoreactive cells in the grafts were identified in each case and rotational behaviour assessed by injection of amphetamine (5mg/kg; i.p.) before grafting and in those animals surviving 30 days. Cell suspension grafts exhibited a VAS slightly in excess of that found in adjacent striatum and HRP leakage in most cases had resolved by day 14. TH positive cells with extensive fiber outgrowth were consistently present in all animals after 14-30 days. In contrast, solid grafts displayed poor VAS in most cases. This was confined to the borders of the graft. MVP was normal by 30 days postgrafting. At 30 days, TH positive cells were either absent or very low in number and situated in the periphery of the graft. The cell suspension technique of grafting offers a higher success rate for viability with a lesser duration of blood-brain barrier dysfunction than do solid grafts. (Supported by the Parkinson Foundation of Canada)

531.13

BLOOD-BORNE ACCESS TO CNS TRANSPLANTS BY ENDOGENOUS PROTEIN AND EXOGENOUS NEUROTRANSMITTER. R.J. Walsh and J.M. Rosenstein, Departments of Anatomy and Neurosurgery, The George Washington University Medical Center, Washington, D.C. 20037.

Previous studies have indicated that barrier properties within the neovasculature of CNS grafts may be altered such that exogenously administered protein such as HRP may infiltrate the neuropil. To further examine this system, non-injected rats bearing cortical or nigral transplants (10 days-1 year postoperative) were perfused with 4% paraformaldehyde and examined immunocytochemically for endogenous rat serum albumin (RSA). The distribution of anti-RSA with regard to the sources and extent of permeability mimicked that of injected proteins; permeation was consistent in ventricular grafts and considerably less and variable in intraparenchymal grafts. Where the allografts appeared to be immunologically rejected, anti-RSA was prominent for months. To examine access of a neurotransmitter, ^{3}HGABA (250 µCi) was systemically administered for 30 minutes. Portions of ventricular grafts were inundated and neurons avidly sequestered the blood-borne transmitter which normally never crosses the blood-brain barrier. The results confirm that barriers may be altered and CNS grafts can be exposed to endogenous protein and administered neurotransmitters such as GABA (NS-17468).

531.14

BLOOD-BRAIN BARRIER PERMEABILITY TO A PERIPHERAL ACETYLCHOLINESTERASE INHIBITOR FOLLOWING FETAL TISSUE TRANSPLANTS INTO ADULT RAT STRIATUM. K.J. Bertram*, S.Y. Lu, A.B. Norman, E.C. Somoza, M. Ennis, M.T. Shipley, and P.R. Sanberg. Division of Neuroscience, Depts. of Psychiatry, Physiology, and Anatomy, University of Cincinnati, College of Medicine, Cincinnati, OH 45267.

The post-transplantation status of the blood-brain barrier (BBB) remains a controversial issue. When treating neurodegenerative disorders, a compromised BBB following neural transplantation could disrupt the homeostatic environment of the CNS. To determine the integrity of the BBB following neural transplantation, unilateral 16-18 day fetal striatal transplants (1-1.3mm3) were placed stereotaxically into the striatum of adult male Sprague-Dawley rats (190-220g). Sham operated rats received equivalent amounts of vehicle. At one week post-transplantation BBB permeability to phospholine iodide (PI), an irreversible acetylcholinesterase inhibitor which normally does not cross the BBB, was examined. Half of the rats from each treatment group were injected with PI (65ug/kg i.m.) 30 minutes prior to cardiac perfusion with a paraformaldehyde/glutaraldehyde fixative. Brains were sectioned (40um) on a microtome and stained for cresyl violet and acetylcholinesterase. In a control experiment, PI (32.5ng) was injected unilaterally into the striatum of normal adult rats 30 minutes prior to perfusion. There was a marked inhibition of acetylcholinesterase in the injected striatum. In rats which received transplants or sham operations, no penetration of systemically administered PI into the transplant or the host striatum was detected. It was concluded that the BBB within the transplant and the host was intact because the BBB was not permeable to PI via the fetal striatal transplants.

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531.15

CONCENTRATION DEPENDANCE OF PHOTORECEPTOR CELL RESCUE BY RPE TRANSPLANTS. L. Li and J. E. Turner Dept. of Neurobiology and Anatomy, Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, NC 27103

We have reported previously that photoreceptor cells are rescued by retinal pigment epithelial (RPE) cell transplants in RCS dystrophic rats (Li and Turner, Exp. Eye Res., 1988). Further studies showed that healthy RPE cells were required for the long term rescue including outer segment integrity. However, vehicle injections initiated a transient, local beneficial effect which diminished with time. In this study, different concentrations of RPE cells were injected into the subretinal space of RCS rats. When analyzed at 1 and 2.5 months after transplantation photoreceptor cell rescue was seen in all groups. However, the results revealed a concentration dependence and saturation of this effect. Therefore, we conclude that healthy RPE cells are required for long term rescue of photoreceptor cells and this rescue is concentration dependent and saturable which is not the case with vehicle injections. This research was supported by grants from NIH (EY04377-08), the National Retinitis Pigmentosa Foundation and Retinitis Pigmentosa Intl.

DEVELOPMENT AND PLASTICITY—VISUAL SYSTEM: RETINOTECTAL CONNECTIONS

532.1

DEVELOPMENT OF THE RETINOTECTAL PROJECTION OF NASO-VENTRAL QUARTER EYES IN XENOPUS LAEVIS. N.Degen,L.Peter* and K.Brändle*.

Zool.Inst.,Univ.Frankfurt,Siesmayerstr.70,D-6000 Frankfurt 11,West Germany.

According to Sperry's chemoaffinity hypothesis (Sperry,R.W., Proc.Natl.Acad.Sci.USA 50:141,1963) the projection of a small eye fragment with a reduced amount of optic fibers should be restricted to that position in the tectum corresponding to its own specificity. In order to verify this assumption, we removed three quarters of the eye anlage in Xenopus embryos of tail bud stages. The remaining quadrant originated from the nasoventral (NV) retina. Beginning with stage 45 up to metamorphosis we examined histologically the retinal projection to the midbrain. Despite the developmental stage of the larvae the projection field of the NV-eyes was always confined to the rostro-lateral part of the tectum. Since quarter eyes seem to retain their original specificity (Degen,N. and Brändle,K. Acta Biol.Hung. 39:191,1988) it can be excluded by this results that fibers of a quarter eye initially occupied an area corresponding to their own specificity. Electrophysiological recordings in the tectum revealed that despite the abnormal location of the projection field, the optic fiber terminations of the NV-eyes are retinotopically organized. Our results indicate a selfassembling of ingrowing optic fibers rather than the preexistence of markers in a virgin tectum. This supports the initial establishment of the retinotectal map.

532.2

ACTIVITY DEPENDENT AND INDEPENDENT ASPECTS OF TOPOGRAPHIC MAP FORMATION IN MAMMALS D.K. Simon and D.D.M. O'Leary Departments of Neurosurgery and of Anatomy and Neurobiology, Washington University School of Medicine, St Louis MO 63110

Retinal axons form a highly ordered, topographic map in the superior colliculus (SC) of adult rats. However, developing retinal axons mistarget widely in the SC, resulting in an initially diffuse retinocollicular projection (O'Leary et al 1986 J Neurosci 6:3692; Simon & O'Leary 1990 Dev Biol 137:125). Normally, most mistargeted axons are eliminated by P12. Retrograde labeling suggests that major targeting errors are removed even when retinal activity is blocked (O'Leary et al 1986). Here we use DiI as an anterograde tracer to examine directly the remodeling of rat retinocollicular axons and the focusing of their arbors in the absence of activity. TTX was injected intraocularly daily from P0 - P12. Each injection was 1/3 of the lethal dose for a single injection. The 24 hr duration of the retinal activity block was confirmed by inactivation of the pupillary light reflex. At P10, in addition to the daily TTX injection, DiI was focally injected into peripheral temporal retina, which is retinotopically matched with rostral SC. We find that when retinal activity is blocked, more mistargeted axons remain at P12 than in age-matched controls. Many of these axons extend into the caudal half of the SC and have multiple branches along their length. Some axons arborize at topographically incorrect regions, but a few mistargeted axons extend a collateral branch into the appropriate terminal zone. It is very apparent, though, that a dense zone of overlapping terminal arborizations do form at the appropriate topographic position in rostral SC. This observation indicates that even in the absence of retinal activity many axons are able to arborize at the topographically appropriate region. Therefore, the formation of a topographically appropriate arbor can be governed by activity independent mechanisms. In contrast, the elimination of aberrantly positioned axons and branches is to some extent activity dependent since their removal is deterred (or delayed) by blocking retinal activity.

532.3

FUNCTIONAL DEVELOPMENT OF THE NEONATAL RAT RETINOTECTAL PATHWAY. S.K. Itaya and S. Molotchnikoff. Dept. Biomed. Sci., Univ. So. Alabama, Mobile, AL 36688, and Dept. Sci. Biol., Univ. Montreal, Montreal, Que.

During the first week after birth, the neonatal rat visual system undergoes several major developmental processes, e.g., synaptogenesis and cell death. We investigated whether action potentials or synaptic transmission could play a role in these processes by measuring spontaneous and evoked responses in the developing retinotectal pathway. In preliminary experiments, we studied 16 rat pups from postnatal day 6 (P6) to P14. Glass microelectrodes (NaCl, 1-2 Mohms) were lowered through the superficial superior colliculus while measuring for field potentials, multiunit, or single unit activity. On P6/7, spontaneous activity was rare (n=3 cases). Starting on P8, there was spontaneous activity in collicular neurons (n=12). Evoked action potentials were measured in response to electrical stimulation of the contralateral optic nerve. From P6-P9 (n=7), evoked responses were inconsistent, with latencies of about 35 msec. However, from P10-P14 (n=7), there were action potentials to optic nerve stimulation. The responses were spikes with a latency of about 15 msec and a stimulus threshold of 60 V. Both spontaneous and evoked responses were biphasic, indicating a somatic origin. In a previous abstract (Soc. Neurosci., 1988), we reported that flash evoked responses first appear around P12/13. Our results suggest that the rat retinotectal pathway becomes functionally capable in three stages: 1) spontaneous activity first appears on P8; 2) electrically evoked collicular responses develop on P10; and 3) flash-evoked responses appear on P12/13. This suggests that the retinotectal pathway becomes functional during the second week after birth.

532.4

EM ANALYSIS OF NMDA-TREATED TECTA IN THREE-EYED *RANA PIPIENS*. Lai-Hsing Yen and M. Constantine-Paton, Dept. of Biology, Yale University, New Haven, CT 06511

Previous work in this lab has shown that chronic NMDA application to the striped tecta of three-eyed frogs causes a dramatic decrease in branching within retinal ganglion cell (RGC) terminals (Cline & Constantine-Paton. 1990. J. Neurosci. Vol 10, April). We would like to know whether changes in the distribution, or the amount of synaptic contact supported by individual RGC terminals accompany the pronounced NMDA related changes in terminal morphology.

Elvax slices with 10^{-4} M NMDA were placed on the tecta of young three-eyed post-metamorphic frogs. The elvax causes release of approximately 0.3 % of the total drug amount per day. The animals survived for 4-5 weeks and a few RGCs were labeled with HRP before sacrifice. The tecta were cut into 40μm sections, reacted with DAB, and processed for EM. The morphology of isolated single RGC terminals was reconstructed at the light microscope level. Semi-thick and intervening ultra-thin sections through these terminals are being used to quantify HRP labeled synaptic profiles at the EM level.

We have previously found that the fine structure of tecta perfused acutely with normal saline is indistinguishable from that of tecta perfused acutely with saline containing 33 μM NMDA. Qualitative inspection of the chronically treated striped tecta in this study also showed no signs of neurotoxicity.

Ordering of branches of the reconstructed RGC terminals with the centripetal Strahler scheme showed that the chronically NMDA-treated terminals have a lower branch order consisting of order 1 to 5 as compared to order 1 to 6 of the same type terminals in control tecta. This decrease in branching with NMDA treatment is consistent with our previous finding. In addition we have found more synaptic convergence in NMDA-treated tecta: 3 to 5 pre-synaptic profiles on the same post-synaptic profile are often seen. Quantitation of this observation is now underway. (Supported by NIH grant EY06039)

532.5

PROTEIN COMPONENTS OF THE RETINOTECTAL MAPPING MECHANISM. P. Steen and M. Constantine-Paton. Biology Dept. Yale Univ., New Haven, CT 06511

The NMDA receptor is an integral component of the activity dependent retinotectal mapping mechanism in the frog, *Rana pipiens*. Chronic treatment with the receptor antagonist, APV, disrupts the precision of this map (Cline and Constantine-Paton, Neuron 3:413, 1989) and disrupts eye-specific segregation in three eyed frogs; whereas chronic agonist (NMDA) treatment sharpens this segregation (Cline et al., PNAS, 84:432, 1987). It is our objective to identify other molecular components of this mechanism. Since activation of the NMDA receptor appears to be the first step in a cascade of events, we have chronically treated animals with APV and NMDA (10^{-4}M in ELVAX implants for 4-6 weeks) and examined tectal proteins for response to these treatments. We have previously reported results of two-dimensional gel analysis after such treatments (Steen and Constantine-Paton, Soc. Neurosci. Abstr. 16:1211, 1990). However, since high molecular weight proteins do not focus well in these gels, they were omitted from these analyses. Therefore, we have utilized silver stained SDS-PAGE 7% gels, which were allowed to run until a 116kD prestained standard migrated to the end of the gel. This procedure allowed us to visualize proteins from 116 kD to well over 400 kD.

We have identified two proteins of approximately 240 and 175 kD which increase in abundance following chronic APV treatment, a 185 kD protein which decreases in abundance after APV treatment, and a 150 kD protein which decreases in abundance following chronic NMDA treatment. The 185 kD protein also appears to migrate slightly slower in the APV treated animals, with an increased apparant molecular weight of about 5 kD. We suggest that not only are these changes coincident with changes in the retinotectal map, but that they are a response of integral components of the mechanism which forms and maintains this map. *Supported by EY06039 NIH Grant.*

532.6

Localization of [^{3}H]Nicotine Binding Sites in the Goldfish Optic Tectum and Recovery During Optic Nerve Regeneration. G.T. Prusky. Yale University, Department of Biology, New Haven, CT 06511.

This study applied the techniques of [^{3}H]nicotine autoradiography to the retinotectal system of the goldfish in order (1), to obtain autoradiographic maps of [^{3}H]nicotine binding in the goldfish optic tectum to determine their relationship to previously published reports of [^{125}I]α-BTX binding, and (2), to determine whether [^{3}H]nicotine binding sites are associated with specific phases of retinal regeneration in the optic tectum. [^{3}H]Nicotine binding sites were located almost exclusively in the SO-SFGS region of the normal goldfish optic tectum. These binding sites were dramatically reduced in the tectum following enucleation of the innervating eye, indicating that they had a presynaptic loci on retinotectal fibers. The expression of [^{3}H]nicotine binding sites was also associated with the loss and subsequent regrowth of retinal terminals in the tectum. [^{3}H]Nicotine binding was reduced to enucleation levels within 1 week of the crush and only recovered to pre-crush levels between 12 and 26 weeks following optic nerve crush. These data suggest that the distribution of [^{3}H]nicotine binding sites in the goldfish optic tectum is different from that of [^{125}I]α-BTX labeling, but like [^{125}I]α-BTX binding, is associated with optic nerve terminals. Also, the recovery of [^{3}H]nicotine binding sites was not associated with primary regeneration of optic nerve terminals in the tectum, but rather with secondary or tertiary events after vision was reestablished. G.T.P. was supported by an N.S.E.R.C. of Canada Fellowship.

532.7

PATTERN OF CYTOCHROME OXIDASE ACTIVITY IN THE DEVELOPING CHICK OPTIC TECTUM. W.J. Crossland and J.D. Peduzzi. Dept. Anat. & Cell Biology, Sch. Med., Wayne State Univ., Detroit, MI 48201 and Dept. Physiol. Optics, Sch. Opt., Univ. AL at B'ham, Birmingham, AL 35294.

Cytochrome oxidase (CO) expression in some visual centers has been shown to be correlated with retinal activity levels (Wong-Riley, 1989). Furthermore, during development transient CO activity in some visual areas has been correlated with cell birthdate (Peduzzi, 1987). Is embryonic CO activity closely linked to the arrival of retinal axons or is it an independent cell property (Lachica et al., 1987) correlated with tectal neuronal birthdate? We have investigated this question in the optic tectum of the embryonic chick. The order of tectal laminar neurogenesis in the chick (LaVail and Cowan, 1971) is: First, the deep layers - stratum griseum centrale (SGC) and stratum periventricularis (SP), then the superficial layers - stratum griseum et fibrosum superficiale (SGFS) a-g, followed by the intermediate layers - SGFS h-j. Standard histochemical methods (Wong-Riley, 1979) were used on free-floating frozen sections of aldehyde-fixed brains from E9 to adult chickens. On E9, when the tectum is only 1/2 innervated by the retina, CO activity was heaviest in SP, SGC, and a band within the presumptive SGFS. Little difference was evident between innervated and noninnervated tectal regions even though CO reactive retinal ganglion cells were evident at this age. (The role of visual input in the pattern of CO activity is further explored in the companion study.) From E9-E14, the addition of new CO bands corresponded with further laminar differentiation in the SGFS a-g and, at E14, in the SGFS h-j. By E18, the mature pattern of neuropil and cellular CO reactivity was recognizable (greatest activity in SGC and SGFS a-c & f). Thus, CO activity appears in a complex pattern, resembling the neurogenetic sequence of the tectal laminae rather than initial retinal ingrowth. Supported by EYO4068, EY07093 (W.J.C.) and EY01338, EY03039, RR05807 (J.D.P.).

532.8

CHANGES IN THE PATTERN OF CYTOCHROME OXIDASE ACTIVITY IN THE CHICK VISUAL SYSTEM FOLLOWING DEAFFERENTATION. J.D. Peduzzi and W.J. Crossland. Dept. Physiol. Optics, Sch. Opt., Univ. Alabama at Birmingham, Birmingham, AL 35294 and Dept. Anat. & Cell Biology, Sch. Med., Wayne State Univ., Detroit, MI 48201.

In the visual system of several species, intense cytochrome oxidase (CO) activity is found in particular laminae or structures that receive direct visual input. To determine the extent that CO activity in the chick visual system is dependent on visual input, newly hatched chicks were unilaterally enucleated under deep anesthesia and allowed to survive for a variable length of time. Sections from control and experimental animals were processed in the same solution for identical lengths of time using the CO protocol of Wong-Riley (1979). In normal chicks, strong CO activity was present in all retino-recipient and visuomotor centers. Tectal layers SP, SGC and SGFS (greatest activity in sublaminae a-c and f) were reactive, as noted in our companion study. Most nuclei that connect with the tectum were also heavily labeled. Ten days after enucleation, the intensity of CO activity appeared to be similar in most deafferented and control visual nuclei (including the ventral lateral geniculate and ectomamillary nuclei). The isthmo-optic nucleus (ION), which projected to the retina of the enucleated eye, underwent retrograde degeneration. However, CO activity in sublamina f (retino-recipient, cell-free sublamina) of the tectal SGFS was reduced compared to the control. Similar results were observed a month after enucleation. The lack of dramatic reduction in CO activity in most visual areas of the chick following eye removal suggests that CO activity is more a property of individual cells in a region than the result of afferent activity. However, with the exception of ION, the few changes that did occur were restricted to areas receiving direct visual input. Supported by EY01338, EY03039, RR05807 (J.D.P) and EYO4068, EY07093 (W.J.C.).

532.9

CELL SPECIFIC MONOCLONAL ANTIBODY IN THE GOLDFISH VISUAL SYSTEM. S. Braverman*, S. Sharief*, I. Rappaport* and S.C. Sharma. Depts. Immunol. Micro. and Ophthalmology, New York Medical College, Valhalla, NY.

Monoclonal antibodies made against goldfish tectal tissue labeled specific cell types in the brain. In the tectum, the pyramidal neurons (Type I) whose soma are located in the SFGS were intensely labeled with mAb 2F8. Purkinje neurons in the corpus cerebellum and valvula cerebelli and the oculomotor neurons were also labeled. In addition to the neurons, labeled glia types include: a) Muller cells in the retina, b) oligodendrocyte-like cells in the optic nerve and c) radial glia in the tectum. No labeled glia were observed in other regions of the brain. During development the antigen recognized by mAb 2F8 appeared first on the Muller cells of the retina before hatching and by 11-14 days post hatching within the cells of the tectum, cerebellum and oculomotor nuclei. At present, it is not clear whether the antigen recognized by mAb 2F8 is the same in the different cell types or a single epitope is shared by several antigens. This antibody will be useful for studying the response of tectal and Purkinje neurons to injury and will aid in the isolation of the different cell types in a tissue culture system. Supported by NEI01426.

532.10

LONG TERM SURVIVAL OF CONNECTIONS BETWEEN FETAL EYES SUTURED TO PERIPHERAL NERVES AND TECTUM B. H. Hallas, A. Feller, S. Fettahlioglu, C. Kaiser, L. Nguyen, M. Wells and M. Zanakis. Dept. of Neuroscience, New York College of Osteopathic Medicine, Old Westbury, NY 11568.

Eighteen day embryonic rat eyes (E18) were either sutured to a 3 cm segment of adult rat sciatic nerve or attached with purified gelled cyanoacrylate. In animals that provided the sciatic nerve segment, the left optic nerve was completed transected 2 mm distal to the orbit and the lens removed. The E18 eye/sciatic nerve bridge was then implanted into the host's eye, while the proximal end of the sciatic nerve bridge was inserted through a burr hole in the cranium into the contralateral superior colliculus.

One year post-implantation of the fetal eye/sciatic nerve bridge, a 20% solution of HRP was injected intraocularly. Twenty-four to forty-eight hours post injection, the animals were sacrificed and the fetal eye bridge and brain processed for HRP histochemistry or electron microscopy. At the light microscopic level, HRP was observed in the bridge and superior colliculus while at the EM level, labelled myelinated axons in the bridge and synapses in the superior colliculis were observed.

532.11

EARLY MONOCULAR ENUCLEATION AND GENICULO-CORTICAL TOPOGRAPHY IN THE GOLDEN HAMSTER. A.J.Trevelyan* and I.D.Thompson*. (SPON: Brain Research Association). University Laboratory of Physiology, Parks Road, Oxford OX1 3PT, United Kingdom.

We have used a double-label technique to study mapping precision in the geniculo-cortical projection of normal adult hamsters and those enucleated as neonates. Green and rhodamine fluorescent latex microspheres (Katz and Iarovici, 1990, Neurosci.,**34**, 511-520) were used as the retrograde tracers. Small, discrete injections (150nl) of each tracer were made (through glass micropipettes) into area 17 on both sides, contralateral and ipsilateral to the remaining eye. The labelling in the dorsal lateral geniculate nucleus (dLGN) was studied in serial brain sections under UV epifluorescence. The number of cells labelled from a single injection was very similar in both normal and deafferented nuclei. This suggests that the number of geniculate cells projecting to a unit area of cortex is not altered by enucleation. In the enucleated animal, the dLGN still receiving retinal input has an essentially normal pattern of projection. However, analysis of the distribution of double-labelled cells and of the overlap in the two populations of labelled cells in the deafferented dLGN shows that early removal of retinal input perturbs the development of precision in geniculo-cortical topography.

532.12

THE EARLY DEVELOPMENT OF VISUAL THALAMOCORTICAL PROJECTIONS IN THE GOLDEN HAMSTER. B. Miller, L. Chou,* and B. L. Finlay Department of Psychology, Cornell University, Ithaca, New York, 14853.

Little is known about the development of topographic organization between thalamic projections and their cortical target. In the hamster, the lateral geniculate nucleus is produced between E9.5 and E12.5 (birth is E16). Their axons do not invade their cortical target until around three days after birth. We examined the development of these projections and their interactions with developing cortex. DiI was placed in the dorsal thalamus of fixed brains of E14 and E16 hamster pups. After two months the brains were sectioned with a vibratome at 75-100um and examined with fluorescent microscopy.

The growing axons are fasciculated in part of the internal capsule but do not maintain nearest neighbor relations. Fascicles branch and cross, and fibers leave one fascicle to join another. In E14 animals thalamic fibers have grown through the internal capsule, but only a few extend beyond this to traverse for a short distance under the developing cortical plate. No retrogradely labeled cell bodies are seen in the region of the cortical plate in E14 animals. In E16 animals thalamic fibers have grown beyond the internal capsule and extend well into dorsal regions of the developing cortex. Retrogradely labelled cell bodies are seen scattered within the growing fibers and more superficially in the developing cortical plate. Thus, subplate projections do not reach the thalamus until approximately the same time that the thalamic projections reach the cortex. Supported by NIH R01 NS19245.

532.13

DISTRIBUTION OF FAL-EPITOPES IN DEVELOPING INTERLAMINAR AND LAMINAR REGIONS OF THE MONKEY AND HUMAN DORSAL LATERAL GENICULATE NUCLEUS. J.K. Mai* and Ch. Schönlau*. (SPON: European Neuroscience Association). Dept. of Neuroanatomy, Heinrich-Heine-University of Düsseldorf, D-4000 Düsseldorf, F.R.G.

Paraffin sections of ontogenetic series of 45 monkey brains (Cercopithecus aethiops aethiops L.) and of approximately 100 human brains were obtained from the C.& O. Vogt-collection. We investigated the expression of the carbohydrate epitope 3-fucosyl-N-acetyl-lactosamine (FAL) by means of immunohistochemistry, using the mouse monoclonal antibody anti-Leu-M1, one member of the CD 15 family. The FAL expression within the dorsal lateral geniculate nucleus (dLGN) followed a biphasic course. A first prominent peak was found before and around birth with intense neuropil and astroglial staining in the interlaminar space. This laminar pattern disappeared short after birth and by 10 weeks of age in the monkey and by 6 month in the human the dLGN appeared nearly unstained. Thereafter, increasing numbers of phenotypically different FAL-positive astrocytes became visible in the segregated layers of the dLGN, which resulted in a second pattern of lamination. The findings suggest, that the FAL expression coincides with neuropil development and related functional maturation of the visual system. Supported by grant from the DFG (SFB 200).

533.1

DONOR CLIMBING FIBERS SYNAPSE ON HOST PURKINJE SPINES
K. Kawamura, S. Murase, S.Yuasa and K. Yoshida
Dept. of Anatomy, Sch. of Med. Keio Univ., Tokyo 160, Japan.

In the host rat cerebellum, where the inferior olive and climbing fibers had been destroyed by intraperitoneal injection of 3-acetylpyridine (3-AP), the medullary primordial tissue (from E14-16) containing the inferior olive was grafted. After 3 weeks, climbing fiber type preterminals bearing closely-packed round vesicles were found that established synaptic contacts on dendritic spines of the host Purkinje cells. Quantitative analysis at the ultrastructural level has been carried out. The control was taken from 3-AP treated, non-grafted site. Main results are the followings. (1) The number of typical climbing fiber preterminals increased from 0.3% -0.9% to 5% after grafting, which was statistically significant (p=0.01). (2) The number of typical parallel fiber type preterminals that contained sporadically distributed round vesicles increased from 13% to 27% after grafting, which was also statistically significant (p=0.01). It is considered that competition of searching targets is likely to occur between the donor climbing and the host parallel fibers in the process of re-modelling of the brain.

533.2

A QUANTITATIVE ELECTRON MICROSCOPIC STUDY OF SYNAPSE FORMATION BETWEEN CULTURED CEREBRAL CORTICAL NEURONS.
Masumi Ichikawa, Kazuyo Muramoto*, Kazuo Kobayashi* and Yoichiro Kuroda. Dept., Anat., & Embryol., and Neurochem., Tokyo Metropolitan Inst. for Neurosciences, Fuchu, Tokyo 183, Japan.

Monitoring of the formation of functional synapses among dissociated cerebral cortical neurons in vitro using video-assisted multi-site Ca^{2+} fluorometry has shown that the presence of the ecto-protein kinase inhibitor (K-252b) inhibits synapse formation (Muramoto et al., 1988). We examined quantitatively the effect of K-252b on synapse formation using electron microscopy. Cerebral cortical cells of rat embryo (18 days) were cultured. After 7 days, the cultured cells were fixed and embedded. Ultrathin sections were cut and photographed randomly, and synaptic contacts were counted on the electron micrographs. The examined area covered more than 3800 μm^2 per culture. The density of synapses was 1.35 ± 0.23/190 μm^2 in control. In the presence of 0.8μM K-252b, the density of synapses was decreased to 0.58 ± 0.23. This result shows that synapse formation is inhibited by K-252b and confirms the result of the functional assay using fluorometry. These data support the idea that ATP released from nerve terminals phosphorylates surface proteins involved in synapse fomation.

533.3

ONTOGENY OF CHOLINE UPTAKE SITES AND M1 MUSCARINIC RECEPTORS IN THE BABOON HIPPOCAMPAL FORMATION
In-Hei Hahn, Harvey S. Singer, Larry Walker, Pedro Lowenstein, Paul Slesinger, Donald Price, Joseph Coyle. Johns Hopkins Univ Sch of Med, Baltimore, MD 21205.

The developmental maturation of the cholinergic septohippocampal system in baboon hippocampal (HF) was analyzed using quantitative receptor autoradiography. On frozen sections, from midgestation, newborn, juvenile, and young adult baboons, we used [^{3}H]-hemicholinium-3 ([^{3}H]-HC3) to label the choline uptake carrier site and [^{3}H]-pirenzepine ([^{3}H]-PIR) to label M1 muscarinic receptors. Both were present in CA1 - CA4, dentate gyrus (DG), and subiculum (SUB) at 100 days gestation, with highest densities in CA1 and CA2. Total [^{3}H]-PIR binding increased rapidly during gestation, peaked at birth in all regions except SUB, then declined by adolescence. Between ages 3 to 5 years, densities were stable in CA1, CA2, DG, and SUB. [^{3}H]-HC3 binding, after only modest increases from midgestational levels, tended to peak at birth with only slight alterations into adulthood. [^{3}H]-PIR was always greater than [^{3}H]-HC3 binding. Density in CA1 and CA2 was most intense over the pyramidal layer; in DG, the molecular layer exceeded the granular layer. In baboon HF, although cholinergic uptake carrier sites and postsynaptic receptors are present in an adult distribution by midgestation, binding densities have different developmental patterns.

533.4

AN ANALYSIS OF RECURRENT EPSPS RECORDED IN IMMATURE CA_3 HIPPOCAMPAL NEURONS.
J.W. Swann, and K.L. Smith, Wadsworth Center for Labs and Research, NY State Dept. of Health, Albany, NY

Excitatory synaptic interactions between pairs of CA_3 hippocampal neurons were studied in in vitro slices of hippocampus taken from rats 10-16 days of age. Experiments were performed in the presence of penicillin in order to suppress synaptic inhibition and permit the full expression of local circuit excitatory synaptic interactions. In 4 of 141 pairs of cells monosynaptic interactions were recorded. The unitary epsps were unusually large and prolonged. At membrane potentials between -60 and -70 the average amplitude was $2.78 \pm .46$ mV. Epsps were usually more than 200 msec in duration. The probability of occurrence was also high $0.94 \pm .04$. The decay of the epsps was much slower than that of responses to somatically injected current. Typically the recurrent epsps were not followed by an afterhyperpolarization or undershot as has been reported in CA_3 pyramidal cells from mature animals. This would suggest that the K+ conductance that has been reported to underlie such undershots has yet to appear at this stage in hippocampal development. Presynaptic burst firing results in dramatic facilitation of unitary epsps. Our results suggest that, while recurrent excitatory synapses are present early in postnatal life, the epsps they produce differ in many ways from their counterparts of adulthood. Support NS18309.

533.5

MORPHOLOGIC STUDIES OF CA3 HIPPOCAMPAL NEURONS IN THE DEVELOPING RAT.
C.M.Gómez[1], F.L.Rice[1], K.L.Smith*[2], J.W.Swann[2]. [1]Dept. of Anat, Cell Biol. & Neurobio., Albany Medical College, Albany, N.Y. 12208 and [2]WCLR, NYS Dept. of Health, Albany, N.Y. 12201.

We are studying the morphological substrates which may be related to an increased propensity the immature hippocampus for seizures. CA3 hippocampal neurons from animals of different ages were characterized physiologically in slice preparations and injected intracellularly with biocytin. Preliminary morphometric analysis of serially reconstructed filled pyramidal cells revealed that the most extensive axonal arborizations occur in the second postnatal week as compared to the first or to adulthood. In week two, we observed the most elaborate axonal networks with varicosities (likely sites of synaptic contact) in Stratum oriens (S. oriens). Several axons were also found in S. radiatum but few were seen in the cell body layer. Neurons from the first postnatal week had more restricted processes. Adult neurons had a dramatic reduction in the axonal networks of S. oriens. Some unique cells had pyramidal cell-like bursting characteristics but basket cell-like axonal plexi in the cell body layer and unusual fine axons in S. oriens and radiatum. We postulate that a developmental overproduction of synapses occurs which results in a surplus of excitatory contacts and enhanced seizure susceptibility in week two. Excess synapses regress with maturation imparting decreased propensity for seizure induction.

533.6

ONTOGENY OF DOPAMINERGIC AND GABAERGIC INNERVATION OF THE INTERMEDIATE LOBE OF THE RAT PITUITARY GLAND.
K.A. Gary and B.M. Chronwall. School of Basic Life Sciences, University of Missouri-Kansas City, Kansas City, MO 64108.

Dopamine and GABA are co-localized in axons innervating melanotropes of the intermediate lobe (IL) of adult rat pituitary. Transmitters co-localized in the adult may not appear at the same time during development. To determine the time sequence and spatial distribution of IL innervation, we stained Sprague-Dawley ILs at embryo days (E) 17-20 and post-natal days (PN) 2, 5, 10, and 28 immunohistochemically using dopamine, tyrosine hydroxylase (TH), GABA, and a glutamine decarboxylase (GAD) antisera. TH and GAD localization allows early identification of axonal populations prior to the presence of neurotransmitter in terminals. Demonstration of the synthesizing enzyme and the final neurotransmitter is important to show neuronal synthesis rather than axonal uptake of the neurotransmitter. No immunoreactivity was observed in E 17 through PN 2. At PN 5, TH, dopamine, and GABA immunoreactivities were all demonstrated. GABA distribution at PN 10 was very similar to the pattern of innervation in the adult. Conversely, TH and dopamine concentrations at PN 10 are highest in the caudal aspect of the IL, and gradually decrease as one moves rostrally. By PN 28, the spatial distribution of TH and dopamine is similar to that observed in the adult, but staining intensity is much less than seen in adult IL. These results indicate a possible difference in onset of dopaminergic and GABAergic innervation occurring in the IL during ontogeny.

533.7

POSTNATAL DEVELOPMENT OF MONOAMINERGIC RECEPTORS IN THE PRIMATE NEOCORTEX. M.S. Lidow, P.S. Goldman-Rakic, D.W. Gallager, and P. Rakic, *Section of Neuroanatomy, Yale University, School of Medicine, New Haven, CT 06510*

Quantitative *in vitro* autoradiography was used to determine the postnatal development of D_1 and D_2 dopaminergic, α_1, α_2 and β noradrenergic, and 5-HT_1 and 5-HT_2 serotonergic receptors in prefrontal, motor, somatosensory, parietal association and visual cortex of rhesus monkey. At least two animals have been examined at birth, 1, 2, 4, 8, 12, 36 and 60 months of age. We found that the densities of these monoaminergic receptors undergo remarkable similar course of postnatal changes which take place simultaneously throughout all cortical areas examined. The overall density of receptors (as indicated by B_{max} values of specific radioligands) increases within the first few months after birth, reaches levels approximately two times higher than in adults and then begins to decrease toward puberty. These changes in receptor density correlate well with previously reported developmental changes in the density of synapses on spines in the cerebral cortex of the same species (Rakic *et al. Science* 232:232 '86). In addition, we found that monoaminergic receptors undergo postnatal change in their laminar distribution. For example, the tendency of monoaminergic receptors to concentrate in the upper cortical layers, characteristic for adult primates, is not present during first four month when most of these receptor subtypes display the highest densities in the deep layers. After that, receptor density increases predominantly in supragranular layers, achieving an adult pattern in the majority of cases by the end of the first year. Our findings of virtually isochronic course of development of monoaminergic receptors in anatomically and functionally diverse cortical regions stands in contrast to the traditional view of hierarchical development of the cortical regions. This indicates that the entire cerebral cortex develops as a whole and that the establishment of interneuronal communication in this structure may be orchestrated by a single genetic or humoral signal.

533.8

INDUCTION OF SULFATED GLYCOPROTEIN (SGP-2) GENE EXPRESSION IN THE STRIATUM FOLLOWING CORTICAL DEAFFERENTATION. T. H. McNeill, H.-W. Cheng, M. Lampert-Etchells, C. E. Finch and G. Pasinetti. Andrus Gerontology Center, Univ. of Southern Calif., Los Angeles, CA 90089

The present study was undertaken to identify potential molecular markers that may characterize the cellular mechanisms that promote cell survival and induce synaptic remodelling following neuronal deafferentation. For this study, we used an established rat lesion model in which contralateral corticostriate fibers are induced to sprout and innervate deafferented striatal target neurons following a unilateral cortical lesion. In particular we examined mRNA prevalence and protein content for glial fibrillar acidic protein (GFAP), an established marker for reactive astroglia and sulfated glycoprotein-2 (SGP-2), a putative inhibitor of complement-dependent cytolysis.

We found that mRNA prevalence for both GFAP and SGP-2 in the ipsilateral ST was increased at 3 days postlesion and reached a maximum (5-10 fold) at 10 days postlesion. By 27 days postlesion GFAP and SGP-2 mRNA prevalence were reduced but still elevated over levels found in the intact ST. Changes in mRNA prevalence for GFAP and SGP-2 were correlated with an increase in the density of immunoreactive fibers for both markers in the ipsilateral ST and paralleled the time course required for the homotypic reinnervation of the lesioned ST by axonal fibers from the contralateral cortex. We hypothesize that both GFAP and SGP-2 play important roles in the cellular events associated the phenomenon of reactive synaptogenesis following neuronal deafferentation and that SGP-2 serves to protect striatal neurons from phagocytic attack while reactive astrocytes remove surrounding degenerative axon and dendritic profiles from the lesion site. This hypothesis is based in part on previous studies that have reported that SGP-2 has a 76% amino acid sequence homology with complement cytolysis inhibitor (CLI) and that CLI suppresses the formation of the terminal complement attack complex (C5b-9) on cell membranes.

533.9

ACTIVITY-DEPENDENT RELEASE OF ATP ACTIVATES ECTO-PROTEIN KINASE TO STIMULATE SYNAPSE FORMATION BETWEEN CULTURED CEREBRAL NEURONS. Yoichiro Kuroda, Kazuyo Muramoto*, Kazuo Kobayashi*, Masumi Ichikawa#. Depts. of Neurochem. and #of Anat. and Embryol., Tokyo Metropolitan Inst. for Neurosciences, Fuchu-shi, Tokyo 183, Japan.

Presynaptically released ATP and its break down products, adenosine derivatives inhibit *and* facilitate synaptic transmission in the CNS and have been proposed as the main mediators of activity-dependent synaptic competition in "tracing circuits" for human long-term memory (Y. Kuroda: *Neurochem. Intern.*, 14, 339, 1989). In a functional assay system to measure synapse formation between dissociated CNS neurons, the continuous presence of an ecto-protein kinase inhibitor (K-252b) inhibits the synapse formation *in vitro* (Muramoto et al: *Proc. Japan Acad. Ser.B*, 65, 319, 1988). Extracellular addition of ATP induced phosphorylation of several cell surface proteins in the culture. To demonstrate the role of released ATP in activity-dependent changes of synaptic contacts, we cultured dissociated cerebral cortical neurons with various concentration of ATP for 7 days. ATP significantly stimulated the synapse formation as observed quantitatively by electron microscopy. These data indicate that the activity-dependent release of ATP provides substrate for the ecto-protein kinase to phosphorylate functional proteins on the synaptic membrane, which stimulate sprouting and synapse formation. This could selectively stabilize the "tracing circuits" for human long-term memory.

533.10

GP39 - AN AVIAN HOMOLOGUE OF THE MAMMALIAN SYNAPTOPHYSIN MOLECULE. A.M. Cunningham* and P.L. Jeffrey, Children's Medical Research Foundation, Sydney, NSW, Australia, 2050.

Synaptophysin (p38) is the major integral membrane protein of small synaptic vesicles. We have characterized a glycoprotein with an apparent molecular weight of 39 kD, designated GP39, from chick brain using a monoclonal antibody. Purification of the protein from chick forebrain by immunoaffinity chromatography and N-terminal sequence analysis showed GP39 to be a homologue of mammalian synaptophysin.

Our immunohistochemical results indicate that similarly to synaptophysin, GP39 was present in virtually all nerve terminals studied in addition to adrenal medullary cells. The pattern of immunoreactivity in the brain, spinal cord, retina and neuromuscular junction was consistent with that reported for synaptophysin and supports the belief that this is an abundant component of small neurosecretory vesicles regardless of the neurotransmitter involved. Solubilization experiments confirmed GP39 was an integral membrane component without any tight cytoskeletal associations. It was N-glycosylated and was demonstrated to produce a 34 kD form with N-Glycanase treatment. The glycoprotein was highly susceptible to proteolysis with a tendency to form a 23-25 kD fragment.

The deduced amino acid sequences of mammalian synaptophysin indicate an evolutionarily highly conserved molecule with four transmembrane spanning regions and a novel cytoplasmic domain. Such a structure has been proposed to imply a possible function as an ion channel with the carboxyl cytoplasmic domain perhaps serving as an extravesicular binding site [Sudoff, T.C. et al., *Science* 238:1142-1144, 1987]. Alternatively, it has been suggested to function in calcium-mediated neurotransmitter release. Our studies of the avian molecule aim to define its role in the development and function of the synaptic terminal.

ONTOGENY OF NEUROENDOCRINE SYSTEMS

534.1

DEVELOPMENT OF GAP-43 EXPRESSION IN THE FOREBRAIN OF NORMAL MICE AND LITTER MATES WITH NEONATAL LESIONS OF THE BASAL FOREBRAIN. C. F. Hohmann, G. Capone, R. L. Neve, L. I. Bennowitz and J.T. Coyle, Depts. of: Psychiatry and Pediatrics, JHU School of Med., Baltimore, MD; Psychobiol., UC Irvine, Irvine CA; Psychiatry, Harvard Med. School, Boston MA.

GAP-43 is a neuronal phosphoprotein associated with axonal growth during development, plasticity and regeneration. Studies in various species have shown that both GAP-43 mRNA and protein are expressed at high levels early during postnatal development and decrease precipitously during the course of maturation. The aim of the present experiments was to closely compare mRNA expression (as visualized by *in situ* hybridization) and appearance of the GAP-43 protein (visualized by immunocytochemistry [IC]) in several forebrain regions during postnatal development of the BALB/CByJ mouse. In addition, we performed neonatal lesions among the cortically projecting, cholinergic basal forebrain neurons [nBM] to determine whether changes in the time course of cortical morphogenesis would alter GAP-43 expression. Such nBM lesions have previously been shown by us to result in a retardation of cortical neuronal differentiation.

Our results confirm that expression of both mRNA and IC for GAP-43 is highest during the first week postnatal, decreased by 2 weeks and is further reduced by one month. However, incongruencies between mRNA expression and IC localization became apparent, both with regard to the pattern of distribution and relative levels between different regions of the forebrain. For example, mRNA expression was higher in cortex and hippocampus than in the striatum at all ages while immunoreactivity appeared higher in the striatum, particularly in adulthood. On the other hand, the ventrobasal complex of the thalamus did not express high levels of either mRNA or IC at any time in postnatal development. Furthermore, we observed GAP-43 expression in cortical pyramidal neurons as late as 1 year postnatal. Finally, neonatal nBM lesions appear to subtlely affect the time course of GAP-43 IR in cell bodies of the ipsilateral cortex and hippocampus.

534.2

NPY Gene Expression in Developing Mouse Brain: An in situ Hybridization Study G. Capone, C. Hohmann, and J.T. Coyle, Johns Hopkins School of Medicine, Depts of Pediatrics, Psychiatry and Behavioral Sciences; The Johns Hopkins University School of Medicine, Baltimore, MD

Previous immunocytochemical studies have shown "NPY like" immunoreactivity throughout the rat brain during development and in adulthood.We have used *in situ* hybridization (ISH) to examine the regional distribution of NPY mRNA in the brains of Balb/CByJ mice at various postnatal ages (including adulthood). A specific, clearly discernable ISH signal was seen overlying discrete cell bodies within the cortex, hippocampus, caudate-putamen, claustrum, olfactory tubercle, endopiriform nucleus, accumbens nucleus, and several thalamic nuclei. On PND 7, signal intensity was highest in cells from the thalamus, caudate-putamen, and hippocampus, and low throughout most of the cerebral cortex. By PND 14, signal intensity increased in the cortex, remained about the same in hippocampus and caudate-putamen, and decreased slightly in thalamus. In the cerebral cortex and several other brain regions, there is an apparent increase in the number and intensity of NPY expressing cells during the first several weeks of postnatal life, relative to adults. The regional anatomic distribution of NPY gene expression is generally compatible with previous immunocytochemical studies performed on adult rat brain. Previously, we have demonstrated a transient increase in the number of somatostatin (Smst) expressing cells in the cerebral cortex following neonatal nbM lesions. Because NPY and Smst are co-localized in many of the same cortical neurons, we are examining NPY gene expression in the cerebral cortex following nbM lesions.

534.3

COMPARISON OF PERINATAL ANGIOTENSIN BINDING IN THE BRAINS OF SHR AND WKY RATS. V.I. Cook, K.L. Grove, J.W. Harding and R.C. Speth. Department of Veterinary and Comparative Anatomy, Pharmacology and Physiology, Washington State University, Pullman, WA 99164-6520.

Angiotensin is best known for its role in blood pressure and electrolyte balance; however, recent experiments have indicated that angiotensin may also have a role in growth and development. Angiotensin can stimulate cell growth, increase release of platelet-derived growth factor, and increase expression of growth-related proto-oncogenes. Therefore, the localization of angiotensin in the brain during early development would be a logical first step in the examination of a possible growth role in the CNS. Of additional interest would be any differential angiotensin growth-related patterns between the spontaneously hypertensive rat strain (SHR) and the normotensive rat Wistar-Kyoto (WKY) that could be indicative of a hypertensive pathogenesis. This study, therefore, utilizes receptor autoradiography to show the localization of ^{125}I-Sar^1,Ile^8-AII receptor binding in the brains of SHR and WKY rats during perinatal development. Specific receptor binding sites were localized and quantified in several angiotensin-related areas of both SHR and WKY rat brains.

534.4

THYROID HORMONE ALTERS THE POSTNATAL DEVELOPMENT OF CHOLINE ACETYLTRANSFERASE (CHAT) IMMUNOSTAINED CORTICAL FIBERS. J.D. Oh, N.J. Woolf and L.L. Butcher. Dept. of Psychology, UCLA, Los Angeles, CA 90024-1563, U.S.A.

The effects of thyroid hormone treatment on the development of ChAT immunoreactive fibers in the cerebral cortex were evaluated. Rat pups were made hyperthyroid by administering 1 mg/kg triiodothyronine (T3) i.p. daily, or hypothyroid by providing 0.3% propylthiouracil in the diet of the dams. Pups were sacrificed at 1, 2, 3, and 4 postnatal weeks. Brain sections were immunostained for ChAT and fiber densities within the frontal, parietal, piriform, cingulate, perirhinal, and retrosplenial cortices as well as the hippocampus, dentate gyrus, and basolateral amygdala were compared between the groups. In the 1 week postnatal brains, faint staining was observed for all groups. At 2 weeks postnatally, more ChAT fibers were present than at 1 week, however, fiber density analyses showed no differences between the hyperthyroid, control, and hypothyroid rat brains. Levels of ChAT fiber staining density increased in the 3-week animals but no further increases were detected in 4 week postnatal brains. At both 3 and 4 weeks, the optical density of ChAT fibers in cortical areas were greater in the T3 treated pups than both control ($p < 0.05$) and thyroid deficient rat pups ($p < 0.01$), and ChAT fibers of hypothyroid pups were were less dense than those in control pups ($p < 0.05$). [Support: USPHS grant NS 10928 to L.L.B.]

534.5

VIP ANTAGONIST PRODUCES NEURONAL DAMAGE AND RETARDATION OF BEHAVIORAL DEVELOPMENT IN NEONATAL RATS. I. Gozes, J.M. Hill, R.F. Mervis, M. Fridkin and D.E. Brenneman. Lab. of Dev. Neurobiol., NICHD, Bethesda, Md. 20892, Peptide Design L.P., Germantown, Md. 20874, Div. Neuropath. Ohio State Univ., Columbus, Ohio 43210, Dept. Organic Chem., Weizmann Institute of Science, Rehovot, Israel.

An antagonist to the vasoactive intestinal peptide (VIP) receptor(s) was synthesized using a hybrid peptide strategy. Previous studies indicated that this antagonist potently inhibited VIP receptor binding, blocked VIP-stimulated cAMP formation in CNS cultures and produced neuronal cell death in spinal cord cultures. The purpose of the present study was to determine if this antagonist would influence neuronal integrity and the behavioral development of neonatal rats, based on the demonstrated neurotrophic action of VIP (PNAS 83:1159, 1986). Rats received daily injections subcutaneously from birth to day 14. Compared with saline-injected controls, cortical pyramidal neurons from the antagonist-injected animals showed widespread dystrophic changes including dendritic spine loss, and coarse, thickened dendrites with grossly abnormal spines. Dendritic branching in such neurons appeared reduced. Observations of developmental milestones/behaviors were made daily, 24 hours after the injection and without knowledge of treatment group. The following were significantly delayed by the antagonist: air righting, negative geotaxis, grasping and fore and hindlimb placing. VIP co-treatment attenuated or prevented the delays. The following were not influenced by the antagonist: surface righting, eye opening, corneal reflex, auditory startle, crossed extensor reflex and cliff aversion. These data suggest that VIP action is important in the development of cortical neurons and complex motor behaviors.

534.6

TRANSIENT INCREASE IN EXPRESSION OF A GAD mRNA DURING POST-NATAL DEVELOPMENT OF THE RAT STRIATUM. K.F. Greif, N.J.K.Tillakaratne, M. Erlander, and A.J. Tobin, Dept. of Biology, UCLA, Los Angeles, CA 90024

We have studied the development of GABA neurons in the rat striatum by measuring levels of GAD mRNA by in situ hybridization, Northern blot analysis, and enzymatic assays. Two genes encode two molecular forms of GAD, GAD_{65} and GAD_{67}, which differ in their dependence on pyridoxal phosphate (PLP), with GAD_{65} more dependent on exogenous PLP for activity. Between P4 and P7, GAD_{65} mRNA increases markedly in the striatum, but not in other regions of the brain. We observe no similar increases in GAD_{67} mRNA. Elevated levels of GAD_{65} mRNA persist for one week and then decline. In parallel with the increase in GAD_{65} mRNA is an increase in PLP-dependent enzyme activity in midbrain homogenates, which include the substantia nigra (SN), the primary projection of GABAergic striatal neurons. We suggest that the postnatal increase in GAD_{65} mRNA is associated with the innervation of the SN by GABAergic neurons of the striatal matrix, which innervate the SN from P3 to P7. The increased expression of GAD_{65} is responsible for the increase in PLP-dependent activity in midbrain homogenates. The selective increase in GAD_{65} may be responsible for increased susceptibility to PLP-antagonist induced seizures after P7. Supported by NS08713 (KFG) and NS22256 (AJT).

534.7

DEVELOPMENTAL REGULATION OF ENDOTHELIN GENE EXPRESSION IN THE HUMAN BRAIN. Alan K.Hall*[1], Michael R.Condon*[1], Stuart D. Cook[2], Lynne H, Parker-Botelho*[3], and Christina Cade*[3].
[1]Urology Research Laboratory,UMDNJ,Newark,NJ,07103
[2]Department of Neurosciences,UMDNJ,Newark,NJ,07103
[3]Department of Pharmacology,Berlex Laboratories Inc,Cedar Knolls, NJ 07927, U.S.A.

The endothelins comprise a family of small, (<2.5Kd), structurally related polypeptides,that exhibit potent vasoconstrictive activity. Other, non-vascular tissues, including the brain, also contain immunoreactive endothelin. We used radioimmunoassays for endothelin (ET)-1 and ET-3 to measure these two isoforms in human brain homogenates at various stages of normal neurogenesis. Low,but detectable (<200fmoles/mg protein) of both proteins were present in fetal brain as early as 47 days post-conception. Neural ET-1 and ET-3 levels increased during gestation and were maximally expressed in the neonatal/adult brain. These findings suggest that the endothelin gene(s) are regulated during human neuroembryogenesis.

(Supported in part by NIH/NCI grant CA49422-01 to A.K.Hall)

534.8

METABOLIC-ENDOCRINE CORRELATES OF THE LATERAL HYPOTHALAMIC SYNDROME:THE FIRST 48 HOURS. L.Bernardis and L.Bellinger. VAMC Buffalo, Sch. of Med., SUNY/Buffalo, 14215 and Dept. Physiol., Baylor Coll. Dent., Dallas, TX, 75246

Mature male rats received bilateral electrolytic lesions (L) in the lateral hypothalamic area (LHA). One group of sham-operated controls was fed ad libitum (CON-ADLIB), a second CON group was pair-fed to the LHA rats (CON-PF). Two days later all rats were killed by decapitation. Body weight, food intake (FI), food efficiency, carcass fat, liver weight, epididymal fat pad (PAD) weight, incorporation of glucose-U-C14 carbon (GLUCINC) into liver total lipid and glycogen, plasma glucose and insulin were significantly (SIG) reduced in LHA vs. CON-ADLIB. Carcass protein, PAD protein and GLUCINC into PAD lipid and glycogen were normal and liver protein and plasma free fatty acids (FFA) were SIG higher in LHA vs. CON-ADLIB. Compared to CON-ADLIB, CON-PF showed the identical changes seen in LHA rats, except that PAD weight was comparable to CON-ADLIB and that CON-PF had SIG less GLUCINC into PAD glycogen than CON-ADLIB. Furthermore, CON-PF had SIG lower plasma glucose, GLUCINC into liver glycogen and PAD total lipid and lower carcass protein than LHA rats. Plasma FFA were SIG higher in CON-PF than in LHA rats. Some metabolic changes in LHA rats appear sooner after LHAL and appear to be food-related. Other changes appear to be independent of reduced FI. Supported by VA and Baylor Coll. Dent. Research funds.

535.1

PRODYNORPHIN mRNA REGULATION BY GABA-A RECEPTOR IN THE MOUSE STRIATUM. C.Jomary[1], J-C. Schwartz* and C. Llorens Cortes*. [1]Johns Hopkins University, Medical School, Molecular Neurobiology Unit, NIDA/ARC, P.O.Box 5180, Baltimore MD21224 USA; Unité de Neurobiologie et Pharmacologie (U109) de l'INSERM, Centre Paul Broca, 2 ter rue d'Alesia, 75014 Paris, FRANCE.

There is clear evidence that striatal GABA receptors control enkephalin levels, release in vitro and in vivo and biosynthesis. Early studies have shown that GABA-A receptor stimulation induced a decrease in striatal enkephalin release and an inhibition of enkephalin gene expression in mouse striatum. In order to know if GABA receptors also modulate dynorphin biosynthesis, we have investigated the effect of GABAergic agents on dynorphin gene expression in mouse striatum. An acute or chronic I.V. administration of muscimol (GABA-A receptor agonist) plus diazepam (benzodiazepine receptor agonist, which potentiates the effect of muscimol) has been tested. Northern blot analysis showed that while striatal prodynorphin mRNA content was not modified after chronic injection of muscimol and diazepam, an increase (1.6X) was observed after acute treatment. The *in situ* hybridization studies, done on half of the same animals treated, supported these observations. These results suggest that muscimol and diazepam have a early stimulation effect (acute treatment) on dynorphin neurons, which is followed by a compensation event (chronic treatment) reflecting an adaptative control of the precursor biosynthesis in dynorphin perikarya. It appears that in the mouse striatum, the GABA system may regulate the expression of preproenkephalin and preprodynorphin genes in a different manner.

535.2

REGULATION OF GLUTAMIC ACID DECARBOXYLASE (GAD) EXPRESSION IN DEVELOPING MOUSE BRAIN: EMBRYONIC TRANSCRIPT CODES FOR A TRUNCATED GAD PROTEIN. G. Szabo*, Z. Katarova*, Zs. Urban*, T.J. Gorcs† and R. Greenspan. *Dept. of Biochem., BRC, Szeged P.O. Box 521, H-6701, Hungary; †Neuromorphology Lab., Semmelweis Med. Univ., Budapest H-1094, Hungary; Dept. of Neurosciences, Roche Inst. of Molec. Biol., Roche Research Center, Nutley, NJ 07110

Two major GAD transcripts are present in developing mouse brain (3.7kb and 5.7kb) as early as embryonic day 13. The 5.7kb message is predominant in embryo and virtually undetectable after birth. The quantity of the 3.7kb transcript increases only by 50% and reaches its adult level by birth. On the other hand the adult forms of GAD protein (59 kDa and 62 kDa) are hardly detectable until the end of the first postnatal week. At the same time a 40 kDa protein can be detected by immunostaining of Western blots of brain extracts from ages E13 to P14. Recently we have identified 2 GAD genomic loci in the mouse Gad-1 and Gad-1ps on Chr 2 and 10 respectively. Gad-1ps by many criteria resembles a processed pseudogene which has a moderately and highly homologous region with mouse GAD cDNA separated by an inframe stop codon. This indicates that Gad-1ps might have arisen from a different transcript expressed very early in development. A MET following the stop codon could serve as a very strong potential initiation site for the translation of the rest of the ORF coding for a 45 kDa protein which is in good agreement with the size of the embryonic form of GAD. Based on our results we propose that a transcript different from the adult one codes for a truncated embryonic GAD protein.

535.3

HALOPERIDOL TREATMENT INCREASES D_2 DOPAMINE RECEPTOR PROTEIN INDEPENDENT OF RNA LEVELS IN MICE. J. R. Goss, A. B. Kelly, S. A. Johnson*, and D. G. Morgan. Andrus Gerontology Center, U.S.C., Los Angeles, CA 90089-0191.

Haloperidol and other antipsychotic drugs require 1-2 weeks before they ameliorate some of the symptoms of schizophrenia. Rats treated with these drugs develop increased numbers of D_2 dopamine receptors. The mechanism involved in this receptor up-regulation is unknown but may result from: a) an increased synthetic rate caused by elevated steady state RNA levels, or b) reduced internalization/degradation due to decreased agonist activation. We treated fifty C57Bl/6J male mice with 3mg/kg/day of haloperidol in their drinking water for 7 days, with no withdrawal period. Haloperidol treatment increased D_2 receptor density (B_{max}) measured with [^{3}H]spiperone by 20% with no change in the K_d (n=8). Northern blot analysis (n=6) failed to detect any difference in D_2 receptor RNA between treated and untreated mice, although differences were easily detected when 10% more RNA was intentionally added to the gels. Prepro-enkephalin RNA increased in the haloperidol treated mice, as described by others previously. These results indicate that increased steady-state RNA levels are not necessary for receptor up-regulation. Supported by NIA Training Grant AG00093 (JRG, ABK); the Greenwall Award from AFAR, AG07892 and an Est. Invest. Award (Am Heart Assn) to DGM.

535.4

ANTISENSE OLIGONUCLEOTIDE INHIBITS EXPRESSION OF ACETYLCHOLINE RECEPTORS. J.P.Alsobrook II. Child Study Center, Yale University School of Medicine, New Haven, CT 06510.

In a preliminary effort to understand the transcriptional regulation of nicotinic acetylcholine receptor (nAChR) expression, a non-ionic antisense oligonucleotide was used to inhibit transport and/or translation of the mRNA coding for the four nAChR subunits.

Expression of nAChRs in muscle cells is regulated at the level of transcription of its four subunits, and can be modeled in BC3H1 cells, a myogenic line which expresses nAChRs when incubated in low serum media. To inhibit this expression, a 12-mer antisense methylphosphonate oligonucleotide (Marcus-Sekura,C., *Anal.Biochem.* 172:289-295, 1988) was designed using a "universal" nAChR cDNA sequence as a guide (Buonanno et al., *J.Biol.Chem.* 261:16451-16458,1986) . Mitotic BC3H1 cultures were incubated in low serum in the presence of 0.4uM and 4.0uM oligonucleotide. Surface nAChRs were assayed at 2.5 days and 5 days using ^{125}I-labeled bungarotoxin. The lower oligo concentration had no effect compared to control, while the higher concentration reduced surface nAChRs by approximately 50%. Subunit-specific antisense oligonucleotides will provide a new tool for analysis of the regulation of nAChR transcripts.

535.5

RAPID QUANTITATION OF TYROSINE HYDROXYLASE (TH) mRNA BY SOLUTION HYBRIDIZATION (SH). Y.S. Zhu, S.B. Jones*, A.D. Branch*, H.D. Robertson*, S.O. Franklin* and C.E. Inturrisi. Cornell Univ. Med. Coll. and Rockefeller Univ., New York, N.Y. 10021.

To facilitate the study of the regulation of TH gene expression, a rapid, sensitive and quantitative method for measuring TH mRNA levels was developed. The assay is based on ribonuclease protection of a 32P-labeled riboprobe (from a 380 bp cDNA of the rat TH mRNA sequence). After hybridization for 16 hrs at 75°C, resistant riboprobe is precipitated with TCA and filtered using a cell harvester. Analysis was by scintillation counting and quantitation of mRNA was by use of a standard curve generated from a TH sense transcript. A novel feature of our approach is the concurrent use of an SH assay which uses a probe for 18S rRNA to calculate total cellular RNA present in each sample subjected to the TH mRNA assay. The hybridization conditions produced a linear curve from 2 to 125 pg of TH sense transcript (equivalent to 10 to 625 pg of TH mRNA). Reserpine treatment (4 mg/kg sc, once daily for two days), produced at 24 hours after the second dose a 4-fold increase in adrenal TH mRNA (4.8 to 21.3 pg/ug RNA) in Lewis rats, a 4-fold increase in SD rats (5.3 to 21 pg/ug RNA) and an 8-fold increase in Syrian hamsters (4.5 to 36.8 pg/ug RNA). These results demonstrate the sensitivity and utility of this method for quantitation of TH mRNA. (Supported in part by NIDA Grants DA-01457 and DA-05130.)

535.6

APPEARANCE OF TYROSINE HYDROXYLASE-LIKE IMMUNOREACTIVITY AND TYROSINE HYDROXYLASE mRNA IN CEREBELLAR PURKINJE CELLS OF THE MUTANT TOTTERING AND LEANER MOUSE. M.C. Austin, L. Abbott †, P. Montpied*, J. Evers*, S.M. Paul*, J.N. Crawley and M. Schultzberg, Clinical Neuroscience Branch, NIMH, Bethesda, MD, †Dept. Vet. Bioscience, University of Illinois, Urbana, IL.

Tottering (tg) and leaner (tg^{la}) are autosomal recessive murine mutations that are characterized by the development, at approximately 4 to 5 weeks of age, of spontaneous absence seizures, focal motor seizures, and ataxia, which continue throughout adulthood. The tg/tg^{la} mutants have considerably more severe focal motor seizures and ataxia compared to the tg/tg mutants. Noebels (1984) reported that the major neurochemical abnormality in the brain of these mice is a noradrenergic hyperinnervation in terminal regions of neurons located in the locus coeruleus (LC). Based upon this finding we examined tyrosine hydroxylase (TH) mRNA levels and TH-immunoreactivity in the brains of these mice. *In situ* hybridization results did not reveal a significant difference in concentration of TH mRNA in LC neurons in tg/tg or tg/tg^{la} mice. However, we now confirm and extend the previous finding by Hess and Wilson (Neurosci Abstr 393.12;1989) of high levels of TH mRNA and TH immunoreactivity (IR) in cerebellar Purkinje cells of these mice. We have found a significant increase in TH grain density and TH-IR in Purkinje cells of young (pre-seizure) and adult tg/tg and tg/tg^{la} mice. Also, Purkinje cells of young and adult tg/+ and +/+ wild type control mice expressed TH mRNA and TH-IR, but at a much lower level. Northern blot analysis confirmed the findings from the *in situ* and immunohistochemical studies. Purkinje cells containing TH mRNA and TH-IR were more numerous in the caudal portions of the vermis and central portions of the flocculus and paraflocculus. These findings indicate that the expression of TH in cerebellar Purkinje cells of tg and tg^{la} tottering mice is independent of seizure onset, and may be related to the genetic defect in this neurological syndrome.

535.7

MECHANISM OF INDUCTION OF mRNA FOR TYROSINE HYDROXYLASE BY MEMBRANE DEPOLARIZATION OF PC12 CELLS.
E.J. Kilbourne and E.L. Sabban. Dept. Biochem & Mol. Biol., N.Y. Med. Coll. Valhalla, NY 10595.

Membrane depolarization is a model of prolonged neuronal activity or stress. We studied the effect of 50 mM KCl, or 150 μM veratridine, on mRNA levels for tyrosine hydroxylase (TH) and dopamine β-hydroxylase (DBH). TH mRNA increased up to five fold after continuous treatment for 1 to 12 hrs with 50 mM KCl. Depolarization with 150 μM veratridine had a similar effect. In contrast, DBH mRNA levels were unchanged by either KCl or veratridine treatment.

The increase in TH mRNA was inhibited by the chelation of calcium with 3 mM EGTA. Although the calcium channel blockers nitrendipene and verapamil inhibited the secretion of norepinephrine from depolarized cells they had no effect on the increase in TH mRNA. Calmodulin antagonists trifluoperizine and W7, which inhibit the depolarization dependent phosphorylation of TH enzyme, had no effect on TH mRNA levels. Lithium (7 mM), an antagonist of the recycling of inositol, alone had no effect. However, when added together, lithium and verapramil prevented the rise in TH mRNA due to membrane depolarization.

We conclude that either extracellular, or IP3 sensitive intracellular, pools of calcium may be required for the induction of TH mRNA in depolarized PC12 cells.

535.8

REGULATION OF TYROSINE HYDROXYLASE GENE TRANSCRIPTION IN PC18 CELLS BY CELL DENSITY.
C. D. Carlson and A. W. Tank, Department of Pharmacology, University of Rochester, Rochester, NY 14642.

Increasing cell-cell contact in rat pheochromocytoma PC18 cells elevates the levels of tyrosine hydroxylase (TH) and TH-mRNA. In previous studies we have shown that these increases are due at least partially to an elevation in the transcription rate of the TH gene. In this report we further investigate the effect of cell density on TH gene transcription. When PC18 cells are cultured at high density (2×10^5 cells/cm^2), the transcription rate of the TH gene is identical to that observed in cells cultured at low density (1×10^4 cells/cm^2) for approximately 6-12 hours. After this prolonged lag period, the transcription rate of the TH gene in high density cells increases 2-3 fold over that observed in low density cells and remains elevated for at least 48 hours. This time course correlates well with that observed for the effect of high cell density on TH-mRNA levels in PC18 cells. In addition, we have examined the role of cAMP in the density mediated induction of TH. The effects of high cell density and the cAMP analog, 8-chloro-4-phenylthio cAMP, are additive on TH-mRNA levels and TH gene transcription rate. These results suggest that in PC18 cells the cell-cell contact mediated stimulation of the TH gene is not elicited by a cAMP-dependent pathway. Furthermore, the time course of this effect on TH gene transcription shows a delayed onset; hence, this effect may depend upon the initial synthesis of factors that regulate the transcription rate of the TH gene.

535.9

HYPOXIA STIMULATES TYROSINE HYDROXYLASE GENE EXPRESSION IN RAT CAROTID BODY.
M.F. Czyzyk-Krzeska, D.A. Bayliss and D.E. Millhorn, Department of Physiology, University of North Carolina, Chapel Hill, NC 27599.

Tyrosine hydroxylase (TH) is the rate limiting enzyme in the biosynthesis of dopamine, a transmitter in the body (CB). The present studies were undertaken to carotid determine if gene expression for TH is increased in CB during hypoxia, a natural stimulus of CB. Adult rats were exposed to either hypoxia (10% O_2) or air (control) for periods lasting from 2 to 48 hr. They were anesthetized and CB, superior cervical ganglion (SCG) and petrosal ganglion (PG) were removed and processed for *in situ* hybridization with [^{35}S]-labeled oligonucleotide probes. TH mRNA was increased at all time points in CB after hypoxia relative to control. In contrast, TH mRNA was not increased in SCG and PG by hypoxia. To determine if increased expression of TH in CB is unique for hypoxia, another group of rats was exposed to hypercapnia (10% CO_2), another natural stimulus of CB. In no case did hypercapnia cause increased expression of TH in CB. Importantly, the increased TH mRNA during hypoxia in CB was unaffected by complete denervation of CB. We conclude that hypoxia, but not hypercapnia, acts directly on CB (*i.e.* does not require neural input) to enhance TH gene expression. (NIH HL33831, HL34919, AHA 881108 and ALA)

535.10

RAT TRYPTOPHAN HYDROXYLASE GENE EXPRESSION IN BRAINSTEM AND PINEAL GLAND.
R.P. Hart, R. Yang* and L.A. Riley. Dept. of Biol. Sci., Rutgers University, Newark, NJ 07102.

We have cloned a segment of the rat tryptophan hydroxylase gene using synthetic oligonucleotides from the cDNA sequence (Darmon et al., J. Neurochem. 51: 312, 1988) as probes. The 15 kb region encodes five exons of the pineal cDNA sequence. Exon sequences are completely homologous to the published cDNA sequence, and the intron-exon junction positions match those predicted by homology with phenylalanine hydroxylase and tyrosine hydroxylase gene sequences.

Using oligonucleotides from the cDNA sequence, we detect two species of mRNA on Northern hybridization (1.8 and 4.5 kb) in both pineal and brainstem RNA. The brainstem TPH mRNA is present at exceedingly low levels--detection was only possible with poly(A)$^+$ RNA and long exposures. However, TPH mRNA was easily detected in pineal total cellular RNA.

In order to begin to determine why such a discrepancy in TPH mRNA levels exists between pineal and brainstem, we utilized our cloned TPH gene sequences in a nuclear run-on assay for transcription. Results indicate similar levels of transcription from the TPH gene in pineal gland, medullary raphe and midbrain raphe regions. This indicates that levels of TPH mRNA are controlled post-transcriptionally. (Supported by NSF BNS 890551.)

535.11

STIMULATION OF PNMT mRNA EXPRESSION BY Ca^{2+} ION INFLUX.
M.J. Evinger, T.H. Joh and D.J. Reis. Dept. Neurobiol. and Lab. Molec. Neurobiol., Cornell Univ. Med. Coll. NY, NY 10021 USA.

Previously we demonstrated that specific neurotransmitters can alter steady state levels of messenger RNA for the epinephrine-synthesizing enzyme phenylethanolamine N-methyltransferase (PNMT). In primary bovine adrenal chromaffin cell culture, PNMT mRNA increases upon treatment with cholinergic (nicotine and muscarine), histaminergic, and imidazole agonists, probably through activation of defined second messenger systems. Because certain neurotransmitter effects are mediated in part by increasing intracellular Ca^{2+} levels, we tested the hypothesis that elevated levels of calcium may independently stimulate production of PNMT mRNA. Accordingly, incubation of bovine chromaffin cells with the Ca^{2+} channel ionophore A23187 or the agonist BAY K 8644 (100 nM) elicited a 2-4-fold increase in PNMT mRNA detected by quantitative hybridization, increases consistent with and of greater magnitude than those produced by 20 mM Ca^{2+}. In chromaffin cells, nicotinic receptors are positively associated with the influx of extracellular calcium. Likewise, blocking Ca^{2+} entry diminishes nicotine-induced increases in PNMT mRNA levels: the Ca^{2+} channel antagonists nifedipine, verapamil and ω-conotoxin as well as EGTA attenuate to varying (40-85%) degrees the PNMT mRNA response to 50 μM nicotine. Considering that Ca^{2+} also stimulates other adrenal medullary genes, notably enkephalin, these results collectively establish a regulatory role for calcium ion influx as an independent and significant neuromodulator of PNMT gene transcription.

535.12

RAT PINEAL/RETINA HYDROXYINDOLE-O-METHYLTRANSFERASE (HIOMT): cDNA ISOLATION, PCR CHARACTERIZATION AND EXPRESSION.
V. Simonneaux and C.M. Craft. Lab. of Mol. Neurogenetics, Psychiatry, UT Southwestern & VA Med. Ctr., Dallas, TX 75235

Melatonin is a unique indoleamine synthesized in pineal and retina from serotonin. Circadian utilization of serotonin during darkness or adrenergic stimulation for melatonin synthesis are achieved through expression of the enzymes arylalkylamine N-acetyltransferase (NAT) and HIOMT. As a first step in the elucidation of the mechanism that regulates these enzymes and the genetic basis of circadian rhythms, we isolated cDNAs which encode these pineal/retinal specific enzymes.

A rat pineal (RP) LambdaZap^{TM} library (Craft et al., J. Neurochem., in press) was screened (1×10^5 plaques) with a cDNA probe for bovine pineal HIOMT (cBPH) (Ishida et al., 1987, JBC 262:2895). Plaque-purified recombinants (cRPH/1-6) were amplified by polymerase chain reaction (PCR) with +SK/-KS cloning site primers. Products were electrophoresed on a 2% agarose gel. Insert sizes were 0.4 to 2.1 kb. Sequence verification of cRPH/1-6 are being analyzed. PCR of the 2.1 kb cRPH paired with +/-BPH primers revealed bands of similar size as cBPH control. PCR of cDNAs synthesized from total rat pineal/retinal mRNA with +/-BPH primers revealed fragments of the predicted size in both tissues with lower levels in retina.

Hybridization of an RNA transfer blot of total RNA electrophoresed on 1.2% denaturing agarose gel with cBPH probe revealed a 2.1 kilobase (kb) mRNA in BP and RP but no detectable signal in retina. RNA blot analysis of mRNAs isolated from RPs throughout the 24 hr cycle with cBPH suggests higher expression of the HIOMT mRNA during darkness. Although HIOMT enzyme activity remains nearly constant thoughout the 24 hr, the mRNA appears to fluctuate. (Support NINCDS/NIH 1R29/NS28126-01, Veterans Admin.)

536.1

Six novel behavioral mutations identified by transpositional mutagenesis in *Drosophila*. L.C. Timpe, T.E. Crowley*, S. Barbel*, Y.-N. Jan and L.Y. Jan. Howard Hughes Med. Inst. and Depts. of Physiology and Biochemistry, UCSF, S.F. Calif. 94143.

In a search for new mutations which affect proteins important for electrical excitability, we screened 3800 mutant lines created by insertion of a P element-derived transposon. The transposon contained the *white* gene and the *E. coli lacZ* gene under the control of a weak promotor. The tissue specificity of *lacZ* expression in these mutants is controlled by the genomic sequences flanking the insertion site (Bier et al., Genes and Development 3:1273, 1989). Screening for embryonic X-gal staining yielded over 1000 lines with CNS patterns; 500 of these were screened for leg shaking under ether. Six autosomal shakers were found, and the transposons were mapped by *in situ* hybridization to larval polytene chromosomes. One mutation is male sterile in addition to being an ether shaker. The sperm from this mutant are immotile when released from the testis into saline culture.

Are the shaking phenotypes due to insertion of the mutagenic transposon? The transposons were excised by a second mobilization, and the revertants scored for shaking. Shaker$^+$ revertants were found for 5 of 6 mutants.

Genomic DNA flanking the sites of the insertion has been obtained by plasmid rescue and by screening a genomic library. This DNA is being used to identify messages on Northerns, for whole mount *in situs* of embryos, and to screen cDNA libraries.

536.2

THE DEVELOPMENT OF ACTIVE MEMBRANE ELECTRICAL RESPONSES AND GLUTAMATE RECEPTORS IN CULTURED RAT CEREBELLAR PURKINJE CELLS. J.R.Brorson[1], J.A. Holzwarth[2], and R.J. Miller[2]. Depts. of [1]Neurology and [2]Pharm. and Phys. Sci., University of Chicago, 947 E. 58th St., Chicago IL 60637.

We have studied the development of active membrane properties of a relatively pure population of cultured Purkinje cells, using whole cell patch clamp and fura-2 based [Ca^{2+}]i microfluorimetry techniques, with a view to elucidate the role of Ca^{2+} in intracellular regulation in these neurons. Cerebella from day 16 embryonic rats were dissociated and cultured in defined serum-free medium over a feeding layer of astrocytes. By immunocytochemical staining for calbindin, which is specific for Purkinje cells within the cerebellum, approximately 90% of cells stained positively. Within the first several days in culture, these cells exhibited voltage activated Na^+ and Ca^{2+} conductances and fired single action potentials. By the end of the first week in culture the conductances were larger and the cells began to fire bursts of Na^+ action potentials during current stimuli. During the second week in culture, trains of spontaneous action potentials became common, similar to the repetitive action potentials reported in Purkinje cells in slices (Kapoor, R. et. al., Neurosci. 26:493-507, 1988). Sensitivity to glutamate, as indicated by a rise in [Ca^{2+}]i, was observed from the earliest times in culture and increased as the cells matured; these responses were greater in Mg^{2+}-free solutions. Thus, these cultured Purkinje neurons show an early progression towards mature cell characteristics.

536.3

DEVELOPMENT OF EXCITABLE MEMBRANE PROPERTIES IN EARLY POSTNATAL RAT SEPTAL NEURONS. J.B. Suszkiw+•, A.E. Schaffner• and J.L. Barker•. •Laboratory of Neurophysiology, NINDS, NIH, Bethesda, MD and +Department of Physiol. & Biophysics, Univ. of Cincinnati Med Sch., Cincinnati, OH.

Septal neurons dissociated from PN1-2 and PN6-7 rats were studied between 24-120 hrs in culture using whole cell patch recording. The neurons had RMPs in the range -40 to -80 mV, resistances 0.5 to 1 Gohms, and 50-90 mV APs. Depolarization from HP -80 mV elicited fast, TTX-sensitive inward (2-3 nA) I_{Na}'s and transient and sustained outward K^+ currents. Pharmacological dissection of the outward currents indicated that by PN6-7 several types of K^+ currents (I_A, I_C, TEA-sensitive and -insensitive I_K) are expressed in various proportions in the septal neurons. Sustained inward Ca^{2+} currents (50-150 pA) were recorded in all PN6-7 neurons tested, but were not routinely detectable in the PN1-2 neurons. All PN1-2 and PN6-7 neurons tested were sensitive to glutamate and GABA but did not respond to ACh or NE. Application of glutamate usually elicited multiple AP's. Preliminary experiments with cell-attached patch recording indicated that like glutamate, GABA also produces depolarizing, functionally excitatory responses. These results suggest that PN1-PN7 septal neurons are still in the process of differentiation of their membrane electrical and chemosensitive properties.

536.4

CALCIUM SPIKES AND CALCIUM PLATEAUX EVOKED FROM DISTAL DENDRITES OF TURTLE SPINAL MOTONEURONES BY APPLIED ELECTRIC FIELDS. J. Hounsgaard* and O. Kiehn*, Inst. of Neurophysiology, Univ. of Copenhagen, Blegdamsvej 3C, DK-2200 Copenhagen N., Denmark.

In motoneurones in transverse slices of the turtle spinal cord nefidipine insensitive Ca spikes are promoted by TEA while nefidipine sensitive Ca plateaux are promoted by 5-HT and apamin (Hounsgaard et al. J. Physiol. 398: 575-589, 398: 591-603, 414: 265-282). We have used differential polarization by applied electric fields (Chan et al., J. Physiol. 402: 751-771 and 409: 145-156) to determine the compartmental origin of the two Ca mediated regenerative responses. During experiments the transmembrane potential was measured at the motoneuronal soma while electric fields were established by passing current between plate electrodes on either side of the preparation. Synaptic responses were minimized by the presence of TTX, 2APV, CNQX bicuculline, strychnine and picrotoxin.

In the presence of TEA electric fields in the ventrodorsal or the mediolateral direction could evoke Ca spikes independent of the polarity of the field and of the membrane polarization at the soma. In the presence of apamin Ca plateaux were generated by the same regime of differential polarization that was used to generate Ca spikes.

The results show that distal dendritesin motoneurones can support Ca spikes and Ca plateaux. This suggests that voltage dependent current generators are involved in local processing of synaptic responses in the dendrites of motoneurones.

536.5

DIRECT DETECTION OF VASOPRESSIN FROM INDIVIDUAL NERVE TERMINALS OF THE RAT NEUROHYPOPHYSIS AFTER WHOLE-CELL PATCH-CLAMP RECORDING. X. Wang*, S.N. Treistman and J.R. Lemos. Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545.

We have developed an immunoblotting technique to detect arginine vasopressin (AVP) in individual isolated nerve terminals from the neurohypophysis. After standard whole-cell patch-clamp recording, the contents of terminals were sucked up into the recording electrode and stored at -20°C. The immunoblot control assays were prepared by blotting known amounts of three antigens (AVP, oxytocin and met-enkephalin) on the nitrocellulose membrane and then exposing to AVP-antibody. AVP showed positive reactions in concentrations ranging from 50 pg - 10 ng, while oxytocin and met-enkephalin showed no reactions. A total of 59 terminals were assayed, 44 of which showed positive reactions and 15 of which showed no reactions. We were also able to characterize the Ca-currents of these individual isolated neurohypophysial terminals. This technique is uniquely designed to assist in direct identification of a variety of antigens and other markers from individual cells (or sub-cellular compartments such as terminals) after whole-cell patch-clamp studies characterizing voltage-gated currents and drug effects, and should allow direct correlation between physiology and cell type. This research supported by PHS grant AA08003.

536.6

ATP RECEPTOR-OPERATED CA INFLUX AND ^{3}H-NOREPINEPHRINE RELEASE IN PC12 CELLS. K. Inoue*, K. Nakazawa, K. Fujimori ,and A. Takanaka, Div. Pharmacology, NIHS, 1-18-1 Kamiyoga, Setagaya, Tokyo 158, Japan

We have previously reported that ATP stimulates ^{3}H-norepinephrine (NE) secretion by a mechanism not coupled with voltage-gated Ca channels (Neurosci.Let.,106,294, 1989). We report here more details of this mechanism as determined using the batch method previously reported (J.Biol. Chem., 263, 8157, 1988), and the whole cell voltage-clamp technique. ATP stimulated intracellular Ca increase and NE secretion from PC12 cells in a dose-dependent manner which paralleled that of the ATP-activated current in the concentration range from 15 μM to 1 mM. The secretion and increase of intracellular Ca were dependent upon extracellular Ca, but were not inhibited by the Ca-channel blockers nicardipine (up to 10μM) or cadmium (up to 300μM). ATP-stimulated NE secretion was not influenced by an increase of extracellular Ca up to 18 mM, but was inhibited by higher concentrations in accord with our report on the ATP-activated Ca current (J.Physiol. (Lond.), in press, 1990). Secretion was inhibited by the P2-purinoceptor blocker suramine (30-300 μM). The rank order of potency of agonists in evoking the secretion was ATP > ATP-γ-S » ADP. β,γ-Methylene ATP, AMP or adenosine had no effect. These findings suggest that extracellular ATP activates P2 receptor-operated Ca channels in PC12 cells and that the resultant Ca influx evokes NE secretion.

536.7

4-AMINOPYRIDINE ALTERS CYCLE FREQUENCY AND PHASE RELATIONSHIPS AMONG PYLORIC NEURONS IN THE LOBSTER STOMATOGASTRIC GANGLION. A.J. Tierney and R.M. Harris-Warrick. Section of Neurobiology and Behavior, Cornell University, Ithaca, N.Y. 14853.

The presence of I_A in certain pyloric neurons (PD,AB,PY) suggests that this conductance could influence cycle frequency and burst phasing of PY cells (Hartline, 1979; Hartline and Graubard, 1984). We used 4-aminopyridine (4-AP; 1 mM) to reduce I_A in cells within the intact circuit and in individual cells isolated from other pyloric neurons. In the intact circuit, 4-AP always increased cycle frequency (X = 27%; n = 8). 4-AP also altered the phase relationships of all cells relative to the PD burst onset. LP, PYs and IC were phase advanced (Xs = 12%, 10%, 6%, respectively, of the total cycle period). The VD was greatly phase delayed (X = 37%), possibly due to enhanced LP inhibition. In all cells, spikes/burst and spike frequency within bursts were significantly increased by 4-AP. Changes in oscillation amplitude were inconsistent, except in the AB neuron where oscillation amplitude always increased during 4-AP application (4-5 mV; n = 4). When cells were isolated from other pyloric neurons (using photoinactivation and PTX; no sucrose block) 4-AP enhanced tonic or bursting activity in all cells. These data are consistent with the hypothesis that cells thought to lack significant I_A (LP, VD, IC) may possess this conductance in nonsomatic cell regions. Supported by NRSA #NS08337-01 (AJT) and NIH #NS17323 (RMH-W).

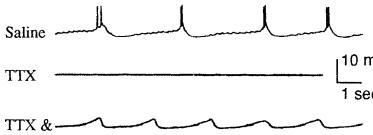
536.8

EFFECTS OF NICKEL ON STOMATOGASTRIC NEURONS. *L. Zirpel, D. Baldwin* and K. Graubard*, Depts. of Physiology & Biophysics and Zoology, Univ. of Washington, Seattle, WA 98195

The divalent cation Ni^{2+} has been shown to specifically block T-type calcium channels in mammalian neurons. However, the effects of this ion on invertebrate neurons are relatively uncharacterized. The objective of this study was to determine the effects of Ni^{2+} on pyloric neurons of the spiny lobster, *Panulirus interruptus*.

The stomatogastric ganglion was maintained *in vitro* at 14°C and perfused with chloride saline. Data were obtained from pre- and postsynaptic somata using 2 two-electrode current clamps. TTX was added to the saline to eliminate spikes and rhythmic activity.

Concentrations of 50-100μM Ni^{2+} induced voltage oscillations of similar amplitude and frequency as the endogenous rhythmic activity. Lower concentrations of Ni^{2+}, 25-50μM, increased the input resistance of the neurons, enhanced both synaptic and electrical transmission and altered the voltage waveforms. These effects are qualitatively different from the simple blocking action of 1mM Cd^{2+}.



These results indicate that Ni^{2+} exerts qualitatively different effects on crustacean and mammalian neurons.

Supported by NS15697.

536.9

CALCIUM DEPENDENCE OF SPIKE REPOLARIZATION IN RAT MAGNOCELLULAR NEUROSECRETORY CELLS (MNCs). **K. Kirkpatrick and C.W. Bourque** ***Centre for Research in Neuroscience, Montreal General Hospital and McGill University, Montreal, Canada H3G 1A4.***

Activity dependent changes in spike duration in MNCs result from the variable expression of a Ca^{++}-component (Bourque and Renaud, 1985, J. Physiol. 363). This study reveals an additional role for Ca^{++} in controlling spike duration. Intracellular recordings were made from 37 supraoptic nucleus MNCs in hypothalamic explants superfused with artificial CSF (32°C). Single spikes were elicited at 0.5 Hz from a fixed subthreshold membrane potential by applying 8 ms depolarizing pulses. Addition of Ni^{++} or Cd^{++} (100-400 μM, n=17) or removal of extracellular Ca^{++} (n=8) consistently and reversibly decreased spike duration. In contrast, raising $[Ca^{++}]_o$ from 2 to 4 mM had either no effect or decreased spike duration (n=4), but increased the rate of spike repolarization. This apparent paradox suggests that Ca^{++} influx may contribute to the activation of repolarizing currents. In agreement, intracellular injection of BAPTA, a Ca^{++} chelator, progressively increased spike duration (n=8) in concert with the disappearance of the AHP. These results suggest that a rapid Ca^{++}-dependent K^+ current may contribute to spike repolarization in MNCs. ***Supported by FRSQ and MRC.***

536.10

MODULATION OF MOTONEURON REPETITIVE FIRING BY MULTIPLE OUTWARD CURRENTS. F. Viana and A.J. Berger. Dept. of Physiology & Biophysics, Univ. of Washington School of Medicine, Seattle, WA 98195.

We examined the firing properties of hypoglossal motoneurons in transverse brainstem slices (500 μm) from mature guinea pigs. Depolarizing constant current pulses from rest (-60 to -70 mV) evoked repetitive firing that showed a decrementing pattern (adaptation). Hyperpolarizing voltage-clamp pre-pulses (1 sec) caused an early decrease in firing rate, which then reversed to an accelerating type of discharge pattern. At slow firing frequencies (<25 Hz) the hyperpolarizing pre-pulse caused a significant delay to the first spike (delayed excitation). Solutions with nominally zero Ca^{2+} and 0.5 mM EGTA (or addition of 300 μM Cd^{2+}) caused an approximate two-fold increase in the slope of the steady-state frequency-current relationship but did not prevent the early decrease in firing and the delayed excitation caused by the hyperpolarizing pre-pulse. The spike duration, including the fast repolarization, was virtually unaffected by removal of Ca^{2+}, but the medium duration afterhyperpolarization was abolished.

In voltage-clamp, after TTX, removal of Ca^{2+} caused a strong reduction of the steady-state outward current evoked by depolarizing voltage steps. Hyperpolarizing pre-pulses resulted in removal of inactivation of a slow transient outward current not blocked in Ca^{2+}-free solution. Both outward currents decreased in amplitude with high extracellular K^+. In some cells, 4-AP (500 μM) caused only modest reduction of the transient current. We conclude that the pattern of discharge of hypoglossal motoneurons is modulated by voltage- and Ca^{2+}-activated potassium currents. These results may prove relevant for aspects of synaptic integration and recruitment timing. (Supported by NS 14857)

536.11

I_H AND RHYTHMIC FIRING IN IDENTIFIED VISUAL CORTICAL NEURONS. J.S. Solomon and J.M. Nerbonne. Dept. of Pharmacology, Washington University Medical School, St. Louis, MO 63110.

We have previously demonstrated that β-adrenergic agonists attenuate a hyperpolarization-activated inward current, I_H, in layer V superior colliculus-projecting (SCP) neurons of rat primary visual cortex. Because norepinephrine disrupts rhythmic firing of lower layer cortical neurons in vivo, we are investigating the role of I_H in patterning the response of SCP cells to inhibitory inputs.

Dissociated SCP neurons from postnatal day 7-13 Long-Evans rats were identified in vitro following in vivo retrograde labeling with rhodamine beads. Whole-cell recordings were obtained within 48 hrs after isolation. In all SCP cells, hyperpolarizations from a holding potential of -40 mV evoke an instantaneous, noninactivating inward current; steps negative to ≈-70 mV also reveal the slowly activating I_H. The current-voltage relation for I_H is linear at potentials more negative than -90 mV (n=5), and I_H is selectively and reversibly blocked by 3 mM extracellular Cs^+ (n=7). The rates of rise of I_H are best fit by single exponentials with mean (±SD) activation time constants of 1.88±0.74 and 0.45±0.22 sec at -80 mV (n=7) and -110 mV (n=11), respectively. Under current clamp, the activation of I_H attenuates the response to hyperpolarizing current pulses. Moreover, deactivation of I_H on removal of the hyperpolarizing current augments the firing of action potentials. I_H, therefore, appears to be important in modulating the efficacy of sustained inhibitory inputs to SCP neurons and patterning cell firing following release from inhibition. (Supported by NSF #BNS 8809823 and NIH #5T32 GM07805).

536.12

DIFFERENT ELECTROPHYSIOLOGICAL CELL TYPES IN ENTORHINAL CORTEX LAYER II IN RAT BRAIN SLICES. A. Alonso and R. Llinás. Dept. of Neurology & Neurosurgery, McGill University, Montreal, Canada, H3A 2B4 and Dept of Physiology & Biophysics, NYU School of Medicine, New York, N.Y, 10016.

Intracellular recordings were obtained from medial entorhinal cortex (MEC) neurons. Two distinct cell types were identified on electrophysiological and morphological basis. The most abundant (65%) cell type (type 1) corresponded to the "large stellete cells" characteristic of MEC layer II (Alonso & Llinas, Nature, 342: 175-177, 1989). These neurons displayed a pronounced time-dependant anomalous rectification (due to a Q-like current) and rhythmic subthreshold Na-dependent membrane potential (Vm) oscillations (7-12 Hz) which persisted the blockage of Ca-conductances. The second cell type (type 2) also had a stellete-like morphology but with one primary dendrite being thicker and longer than the others. Type 2 differed from type 1 cells by their slower action potentials, larger after-hyperpolarizations, weaker IQ "sag" and delayed firing upon depolarization. They also displayed subthreshold Vm oscillations that were less stable and of lower frequency than those in type 1 cells and which demonstrated a dual ionic mechanism being both Na- and Ca-dependent. Type 1 and type 2 neurons could also be distinguished by their sensitivity to cholinergic agonists which induced drastic changes only on type 2 cells membrane properties. In these neurons, in the presence of carbachol (40uM), post-train after-hyperpolarizations were substituted by long-lasting plateau depolarizations that sustained repetitive firing. Moreover, weak constant current injection induced slow rhythmic (< 1Hz) bursting activity. Because of the key anatomical position of EC layer II, these two cellular types will significantly contribute to the network behavior of the hippocampal system and may underlie the generation of the two known distinct types of limbic theta rhythm.

536.13

EFFECTS OF NEOMYCIN AND PHENCYCLIDINE ON TWITCH TENSION AND NERVE TERMINAL CURRENT AFTER POST-TETANIC POTENTIATION. M.C.Tsai and M.L.Chen*. Pharmacological Institute, College of Medicine, National Taiwan University, Taipei, Taiwan, R.O.C.

Post-tetanic potentiation (PTP) is a transiently increased responsiveness of the motor nerve and the increase in amount of transmitter release is responsible for the PTP. In the present study, the effects of neomycin and phencyclidine on the twitch tension and nerve terminal current after tetanic stimulation were studied on the isolated phrenic nerve diaphragm and m. triangularis sterni of mice. Neomycin and phencyclidine were associated with less post-tetanic potentiation of twitch tension if the duration of tetanic stimulation were 10 or 20 sec. Both compounds affected neither the compound action potential of phrenic nerve nor the sodium and potassium currents of the nerve terminal after repetitive stimulation. However, neomycin decreased while phencyclidine does not decrease the calcium current in the nerve terminal after repetitive stimulation. The results suggested that neomycin affected the PTP of twitch tension by pre-synaptic mechanism while phencyclidine affected the PTP by post-synaptic mechanism. (Supported by National Science Council, R.O.C.)

536.14

ION CHANNELS IN PARANODAL SCHWANN CELL MEMBRANES OF ADULT MAMMALIAN MYELINATED FIBERS G.F.Wilson*, W. I. Welker and S.Y.Chiu.Neuroscience Training Program & Dept. of Neurophysiology, University of Wisconsin, Madison, WI 53706.

The intimate association between Schwann cell and axon in the nodal region suggests that this is a likely site for functional specialization. We examine whether there is a localized expression of channels in the Schwann cell paranodal regions using freshly isolated adult rat sciatic nerve fibers. Cell-attached and outside-out patch clamp recordings were made from paranodes where the myelin had been retracted by enzymatic treatment. Although no myelin was visible on the surface of retracted paranodes, significant portions of this surface stained with a marker (anti-galactocerebroside) for Schwann cell membrane suggesting that part of the axon still was covered by glial membrane. Indeed, Lucifer Yellow in recording pipettes diffused either into axons or Schwann cells when the membrane under the pipette tip was ruptured. Using this method to identify the origin of paranodal membranes we found delayed and inwardly rectifying potassium channels on both axon and Schwann patches. Sodium channels, however, were detected only in axon patches. This is the first report that voltage-gated glial channels are present in immediate vicinity to PNS axons. Coupled with earlier reports demonstrating that functional channels are absent in soma of adult myelinating Schwann cells, these results suggest that glial ion channels are regionally specialized for functional interactions with axons.

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536.15

WHOLE-CELL PATCH CLAMP ANALYSIS OF THE PASSIVE MEMBRANE PROPERTIES OF HIPPOCAMPAL NEURONS

N. Spruston & D. Johnston, Division of Neuroscience, Baylor College of Medicine, Houston, TX 77030.

Passive membrane properties are important determinants of electrical responses in neurons with extensive dendritic arborizations. Intracellular microelectrode recordings have revealed that the specific membrane resistivity (R_m) of hippocampal neurons is relatively high. However, it is well known that microelectrode impalement introduces a leak conductance that may affect the passive membrane properties of the cell. We have used patch clamp recording of hippocampal neurons to test the hypothesis that this somatic leak conductance results in an underestimate of R_m.

Hippocampal neurons were acutely-exposed as described by Gray & Johnston (*Nature* 327:620, 1987). Whole-cell patch clamp recordings from dentate granule neurons revealed a rapid decrease of input resistance (R_N) and membrane time constant (τ_m) during the course of the experiment. Since the measured values of R_N and τ_m are directly dependent on R_m, it is likely that the observed rundown is mediated by a decrease in R_m. This rundown could be prevented by including an ATP regenerating system in the pipette or by using perforated-patch recording, suggesting that a conductance mediated by ATP-dependent K^+ channels may develop during cytoplasmic dialysis.

Under conditions where stable recordings of passive responses were obtained, we measured a τ_m of 49±7 ms (mean±SEM; $n = 6$) for dentate granule neurons and 35±6 ms (mean±SEM; $n = 6$) for CA1 pyramidal neurons. These findings suggest that R_m may in fact be higher than previously determined using microelectrodes. Such high values for R_m may serve to enhance the passive propagation of distal synaptic signals to the soma.

(Supported by grants MH44754, NS11535, and AFSOR 88-0142).

536.16

MATHEMATICAL MODELING OF THE SEROTONERGIC MODULATION OF ELECTROPHYSIOLOGICAL PROPERTIES OF SENSORY NEURONS IN APLYSIA. D.A. Baxter and J.H. Byrne, Department of Neurobiology and Anatomy, Univ. of Texas Medical School, Houston, TX 77225.

Serotonin (5-HT) enhances excitability and broadens the spike in the somata of pleural sensory neurons. In order to assess the relative contributions that the serotonergic modulation of three K^+ currents make to the overall effects of 5-HT, we have constructed a Hodgkin-Huxley type membrane model. The model consists of a membrane capacitance, a leakage conductance, differential equations that describe eight membrane currents, and a description of intracellular Ca^{2+}. The parameters were adjusted to simulate the characteristics of the membrane currents, spike, and excitability that are observed normally. The cAMP-dependent actions of 5-HT were simulated by reducing the maximum conductance of the 'S' K^+ current ($I_{K,S}$) and a slow component of the Ca^{2+}-activated K^+ current ($I_{K,Ca}$), whereas the cAMP-independent action of 5-HT was simulated by slowing the kinetics of the delayed or voltage-dependent K^+ current ($I_{K,V}$) (Baxter & Byrne 1989). The simulated effects of cAMP more than doubled excitability but produced only modest spike broadening. The subsequent modulation of $I_{K,V}$ doubled the duration of the simulated spike, without further increasing excitability beyond that produced by the cAMP-dependent modulation of K^+ currents. These results are remarkably similar to the physiological actions of cAMP and 5-HT, and support the hypothesis that the cAMP-dependent actions of 5-HT play a key role in regulating excitability, whereas the cAMP-independent effect of 5-HT has an important role in regulating the duration of the spike. Supported by Grant AFOSR 87-0274.

536.17

A MODEL OF THE CA3 HIPPOCAMPAL PYRAMIDAL CELL BASED ON VOLTAGE-CLAMP DATA. R.D. Traub, R.K.S. Wong and R. Miles. IBM TJ Watson Res. Ctr., Yorktown Heights, NY 10598, Dept. of Neurology, Columbia University CPS, NY, NY 10032, and Institut Pasteur, Paris, France.

It is desirable to have accurate models of CNS cells to understand the role of active membrane currents in synaptic integration and plasticity, and in determining different firing patterns, and to use as building blocks for network models. We have constructed a model of the CA3 cell as a non-uniform cable, with 6 spatially distributed active conductances: g_{Na} (4), $g_{K,delrect}$ (5), $g_{K(A)}$ (3), g_{Ca} (2), $g_{K(C)}$ (1), and $g_{K(AHP)}$ (3). The last 2 currents are Ca dependent; C-current is also voltage-dependent. Kinetics were derived from isolated pyramidal cells; C-current kinetics were derived from frog sympathetic ganglion cells. $[Ca^{2+}]_i$ had 1st order kinetics with removal τ 15 ms. Since the spatial distribution of ionic conductances is unknown, we found a consistent distribution by fitting simulated potentials to current clamp data from isolated cells, whole cells, and dendrites (7). The model then agrees with 2 critical experiments: generation of a depolarizing afterpotential after a stimulus subthreshold for bursting; and a transition from low-frequency bursting to high frequency repetitive firing as somatic membrane potential is raised (6). The model predicts the occurrence of repetitive Na-Ca spikes during steady stimulation of distal dendrites. When model cells are synaptically connected in an excitatory network, afterdischarges occur similar to those recorded in picrotoxin. REFERENCES: 1) Adams PR, Constanti A, Brown DA, Clark RB (1982) *Nature* 296: 746-749; 2) Kay AR, Wong RKS (1987) *J. Physiol.* 392: 603-616; 3) Numann RE, Wadman WJ, Wong RKS (1987) *J. Physiol.* 393: 331-353; 4) Sah P, Gibb AJ, Gage PW (1988) *J. Gen. Physiol.* 91: 373-398; 5) ibid 92: 263-278; 6) Wong RKS, Prince DA (1981) *J. Neurophysiol.* 45: 86-97; 7) Wong RKS, Prince DA, Basbaum AI (1979) PNAS 76: 986-990.

536.18

REDUCTION OF DIMENSION IN DYNAMICAL SYSTEMS THAT DESCRIBE COMPLEX NEURONS. T.B.Kepler, L.F.Abbott and E.Marder, Departments of Biology and Physics and the Center for Complex Systems, Brandeis University, Waltham, MA 02155.

We develop a formal and systematic scheme of coordinate transformations and perturbation expansions for a class of dynamical systems which includes the Hodgkin-Huxley equations and many models of bursting neurons. To zeroth order, one subset of variables effectively decouples from the rest, leaving a system of fewer degrees of freedom, while preserving to the largest extent possible the time-dependent behavior of the membrane potential and conserving stability at the singular points.

When applied to models of neurons containing many conductances, this method provides direct understanding of the mechanisms underlying burst generation and provides computational efficiency when such models are used to construct networks.

Supported by MH 46742 and T32 NS07292.

536.19

Large-Scale Compartment Model of a Cerebellar Purkinje Cell. P.C. Bush and T.J. Sejnowski Salk Institute, La Jolla, CA 92037.

Cerebellar Purkinje cells have a variety of active conductances on both the soma and dendrites. These conductances produce a characteristic firing pattern in response to a depolarizing current pulse injected by a microelectrode at the soma. A computer model of a Purkinje cell has been constructed consisting of over 1000 compartments (Shelton 1986) and seven different conductance types. Instead of using Hodgkin-Huxley kinetics, a multivariable system difficult to apply in the absence of exact voltage-ciamp data, we used a channel model based on Markov chain kinetics, a system with fewer variable parameters that is easy to understand and manipulate.

The model reproduced the firing pattern of real cells in response to current input as reported by Llinas *et al* (1980); A slow depolarization due to sodium and calcium plateau currents causes an accelerating train of sodium-dependent action potentials at the soma. A high-threshold calcium-dependent spike is triggered in the dendrites just at the point of inactivation of the sodium spike train. Voltage- and calcium-dependent potassium currents then produce a large hyperpolarization which reactivates the sodium spikes, allowing the cycle to begin again. This cyclical firing pattern has a period on the order of hundreds of milliseconds, whereas single synaptic potentials last only for tens of milliseconds. Trains of EPSP's are being modelled to establish roles for these conductances under physiological conditions.

$5HT_3$ RECEPTORS

537.1

[3H]QUIPAZINE BINDING SITES IN HUMAN LIMBIC SYSTEM. A. Abi-Dargham*, M. Laruelle, M.F. Casanova*,D.R. Weinberger and J.E. Kleinman, CBDB, NIMH Neuroscience Center, Washington, D.C. 20032

Serotonergic $5HT_3$ receptor blockade antagonizes the secretion of dopamine in rat brain suggesting a potential antipsychotic property of $5HT_3$ antagonists. [3H]quipazine labels $5HT_3$ receptors in rat cortex (Milburn C.M. et al., J. Neurochem., 52:1787, 1989). Displacement of [3H]quipazine binding by the selective $5HT_3$ antagonist ICS 205-930 in human amygdala showed a high affinity site of 0.25±0.03 nM and a low affinity site of 660±130nM. We performed saturation studies with [3H]quipazine (0.25 to 20 nM), using ICS 205-930 (100nM) to define the non specific binding. In the frontal cortex and caudate, no specific binding was detectable. In amygdala, the Ligand analysis of the Scatchard plot was compatible with a one site model (n=3) : Kd= 5.8±0.5nM, Bmax=42.6±5.8 fmol/mg of Protein. Similar results were obtained in human hippocampus. The labelled site in human amygdala and hippocampus is comparable to [3H]quipazine binding site to 5HT3 receptor in rat brain. This confirms the selective limbic localization of these sites in human brain as previously described with [3H]zacopride (Barnes, J.M., J. Neurochem., 53:1787, 1989) and suggests a rationale for the investigation of the antipsychotic properties of $5HT_3$ antagonists.

537.2

IN VITRO INHIBITION OF K^+-EVOKED NOREPINEPHRINE RELEASE BY 5-HT3 RECEPTOR ACTIVATION. P. Blandina, J. Goldfarb, J. Walcott* and J. P. Green. Department of Pharmacology, Mount Sinai School of Medicine, City University of New York, New York, NY 10029 , U.S.A.

There is much evidence for 5-HT /NE (norepinephrine) interactions in the C.N.S. We show here that 5-HT modulates the release of endogenous NE. Hypothalamic slices (400µ) from adult, male Sprague- Dawley rats were superfused with a medium containing, in addition to the usual salts and glucose, nomifensine (10µM), l-tyrosine (50µM) and pargyline (10µM). NE in the effluent was extracted with alumina and measured by HPLC with electrochemical detection. Spontaneous release averaged 0.25 ± 0.03 pmol/mg protein/3 min (n=48).Two identical 20 mM K^+stimulations, given at a 54 min interval,each almost doubled NE release in a Ca^{2+} -dependent manner. 5-HT (1-10µM) and 2-CH3-5-HT (3-10µM) inhibited K^+-evoked release up to about 50%, the first only in the presence of 5-HT1-like/5-HT2 antagonists (ritanserin, methysergide, 1µM). Nanomolar cocentrations of the 5-HT3 antagonists ICS 205-930 and (-)zacopride (zac) reduced the effect of both agonists. (-)Zac was 10 times more potent than its (+)isomer. (-)Zac has a 10-25 times greater affinity for 5-HT3 receptors than (+)zac (Pinkus, L.P., Gordon, J., Sarbin, N. and Barefoot, D. (1989). Int. Symp. on Serotonin from Cell Biology to Pharmacology and Therapeutics,Florence,3/29-4/1,p.71.). Supported by a grant (DA 01875) from N.I.D.A.

537.3

BLOCK OF CISPLATIN-INDUCED EMESIS BY SEROTONERGIC ($5HT_3$) ANTAGONISTS IN SUNCUS MURINUS. N.Matsuki*, Y.Torii, M.Muto and H.Saito. Dept. of Chem. Pharmacol., Fac. of Pharm. Sci., Univ. of Tokyo, Tokyo 113, Japan.

We have shown previously that the Suncus murinus (house musk shrew), a species of insectivora, vomits in response to various emetic stimuli including cancer chemotherapeutic agents. In the present study emetic responses induced by cisplatin were characterized and effects of anti-serotonergic drugs were studied. Intravenous or intraperitoneal injection of cisplatin elicited vomiting responses dose-dependently. The ED_{50} values for intravenous and intraperitoneal administration of cisplatin were 8.4 and 10.0 mg/kg, respectively. Vagotomy completely abolished cisplatin-induced emesis. Di-aqo complex (DAC), which is considered as active form of cisplatin, similarly induced emesis with significantly shorter latency.

Selective serotonergic $5HT_3$ antagonists (ICS205-930, zacopride, BRL43694, GR38032F) strongly prevented cisplatin-induced emesis whereas the drugs were ineffective against veratrine, copper sulfate and motion stimulus. Intravenous or intraperitoneal injection of serotonin caused vomiting which was prevented by ICS205-930 and vagotomy. These results suggest that cisplatin is converted to DAC and releases peripheral serotonin which subsequently induces emesis through peripheral $5HT_3$-receptors.

537.4

2-METHYL-BUFOTENIN AS A SELECTIVE SEROTONIN (5-HT_3) RECEPTOR LIGAND. S.K.Long*, W.C.M.Cramer*, M.Th.M.Tulp* and B. Olivier. DUPHAR B.V., P.O.Box 900, 1380 DA Weesp, The Netherlands.

We have previously described the action of bufotenin, at the 5-HT_3 subclass of serotonin receptors. In functional assay systems bufotenin acts as a partial agonist (Cramer et al. IUPHAR, 1990). In this study we have pharmacologically profiled 2-methyl-bufotenin (2-Me-BUFO).

2-Me-BUFO showed marginal selectivity for 5-HT_3 receptors, over 5-HT_{1A}, 5-HT_{1B}; 5-HT_{1C}, 5-HT_{1D} and 5-HT_2 receptors in conventional ligand binding assays. K_i values were, respectively : 470, 1500, 3630, 535, 625, 4360 nM. 5-HT has previously been shown to depolarize vagal nerve fibres in vitro at an action at 5-HT_3 receptors (Ireland,S.J. and Tyers,M.B., Brit.J.Pharmacol., 90:229, 1987). However when assayed as a depolarizing agent, 2-Me-BUFO was only a weak partial agonist in comparison to 5-HT. The depolarizing effect of 2-Me-BUFO was sensitive to antagonism by ondansteron (GR 38032F); log K_b = 8.92 ± 0.07 (4). Further 2-Me-BUFO (1 µM) was capable of antagonizing the depolarizing response to 5-HT; log K_b 6.10 ± 0.06 (4).

In conclusion although 2-Me-BUFO shows some specificity for 5-HT_3 receptors, it is unlikely to be a useful 5-HT_3 receptor specific agonist.

537.5

^{3}H-GR 65630, ^{3}H-BRL 43694, AND ^{3}H-QUIPAZINE BINDING TO BOVINE AREA POSTREMA 5HT$_3$ RECEPTORS: AN IMPROVED RADIOLIGAND BINDING ASSAY. M. Teitler and S. Le Page*. Dept. of Pharmacology and Toxicology, Albany Medical College, Albany, N.Y. 12208

Brain 5HT$_3$ receptors have been detected using radioligand binding methodology (see ref.1). However the specific signal produced has either a) not been of sufficient reliability to allow routine screening of drugs or detailed examination of the molecular characteristics of 5HT$_3$ receptor/drug interactions, b) requires a radioligand not commercially available, or c) requires excessive amounts of brain tissue to be practicable. A recent autoradiographical study revealed a high density of 5HT$_3$ receptors in human area postrema (1). Therefore we decided to investigate the possibility of developing a reliable 5HT$_3$ receptor radioligand binding assay in bovine area postrema homogenates.

Radioligand (1nM)	Bound (pmol/g)	%Specific
^{3}H-GR 65630	3.0	82
^{3}H-BRL 43694	2.1	77
^{3}H-Quipazine	0.5	36

The preliminary data presented in the table were derived using 3 mg wet weight bovine area postrema homogenates and 10^{-5}M ICS 205930 as the non-specific determinant. The high levels of binding and the high % specific binding obtained using ^{3}H-GR and ^{3}H-BRL indicate that the assay using these radioligands should be highly reliable; the use of bovine tissue and commercially available radioligands (New England Nuclear) greatly increase the convenience of assaying the 5HT$_3$ receptor on a routine basis. A detailed characterization of the bovine 5HT$_3$ receptor will be presented.

1. Waeber,C.,Hoyer,D. and Palacios,J.M. (1989) Neuroscience,31,393-400

537.6

ML-1035, A SPECIFIC 5HT3 ANTAGONIST, FACILITATES GASTRIC EMPTYING BY ACTIVATING MYENTERIC CHOLINERGIC NEURONS. MD Linnik, BT Butler*, RR Gaddis*, NK Ahmed* Dept of Pharmacology, Marion Merrell Dow Inc, Kansas City, MO.

Certain benzamides facilitate gastric emptying by modifing neurogenic activity in myenteric plexus. This report describes the pharmacology of ML-1035, a highly site-specific benzamide, in studies designed elucidate the neurogenic mechanisms underlying benzamide-induced prokinesis. The following evidence indicates that the activity of ML-1035 is specific for 5-HT3 receptors. In radioligand binding assays, ML-1035 readily displaced a 5-HT3 specific ligand, 3H-GR 65630, from rat entorhinal cortex membranes (Ki = 0.131 uM). In contrast, ML-1035 had a Ki > 10 uM at 9 other receptor sites, including 5-HT1, 5-HT2 and DA2 receptors. ML-1035 also dose-dependently inhibited the bradycardia induced by IV bolus injections of 5-HT to anesthetized rats (IC50 = 17 ug/kg/ IV). *In vivo*, ML-1035 enhances gastric emptying of a semisolid, caloric meal in the rat (EC50 = 3.1 mg/kg, ip). *In vitro*, ML-1035 elicited a dose dependent contraction in guinea pig ileum (EC50 = 15.5 uM). This contraction could be blocked by tetrodotoxin (1 uM) and by atropine (1-3 uM), suggesting that the contraction is the result of indirect activation of cholinergic fibers. Therefore, these data demonstrate that ML-1035 is a highly specific 5-HT3 ligand which enhances gastric emptying by facilitating cholinergic transmission in myenteric neurons.

537.7

5-HT$_4$-LIKE RECEPTORS MEDIATE SLOW EXCITATORY RESPONSES TO SEROTONIN IN THE CA1 REGION OF THE HIPPOCAMPUS. Y. Chaput and R. Andrade. Dept. of Pharmacol, St. Louis Univ. School of Medicine, St. Louis, MO 63104.

We have previously shown that 5-HT, acting on receptors that do not belong to either the 5-HT$_1$, 5-HT$_2$ or 5-HT$_3$ subtypes, elicits a a marked reduction of calcium-activated afterhyperpolarization (AHP) present in CA1 hippocampal neurons. We have now used intracellular recording techniques in *in vitro* brain slices to further characterize the pharmacology of this response.

Bath administered 5-HT dose-dependently reduced the AHP in the range of 1-100μM with an EC50 of 3-10μM while 5-methoxytryptamine and 5-carboxyamidotryptamine were less potent than 5-HT. In contrast, the 5-HT$_3$ receptor agonists 2-methyl 5-HT (30μM) and phenylbiguanide (30μM) were without activity at this receptor. Administration of the gastrokinetic benzamides BRL 24924 (1-30μM), cisapride (1-30μM) and zacopride (30μM) blocked the effect of 5-HT on the AHP without altering the AHP themselves, while the neuroleptic benzamide sulpiride was without effect.

These results support previous studies suggesting that the receptor mediating the decrease in the AHP in the hippocampus does not belong to he 5-HT$_1$, 5-HT$_2$, or 5-HT$_3$ subtypes and demonstrates the existence of 5-HT$_4$-like receptors in the adult rat CNS capable of mediating slow excitatory responses to 5-HT. Supported by Grant MH43985, an MRC postdoctoral Fellowship to Y.C and the Sloan Foundation.

537.8

SEROTONIN 5-HT3 ANTAGONISTS FAIL TO INFLUENCE THE INTRAVENOUS SELF-ADMINISTRATION OF COCAINE OR AMPHETAMINE BY LABORATORY RATS R. Peltier and S. Schenk, Texas A&M Univ., Dept. Psychol., College Station, TX, 77843.

It has been suggested that 5-HT3 receptor antagonists may be useful in the treatment of drug abuse. To assess this possibility, we compared the effects of two antagonists, ICS 205-930 (0.01, 0.1 or 1.0, mg/kg, IP) and GR38032F (0.01, 0.1 or 1.0 mg/kg, IP), with the specific D2 dopamine receptor blocker, haloperidol (0.125 mg/kg, IP), on the intravenous self-administration of cocaine (0.5 mg/kg/infusion) or amphetamine (0.05 mg/kg/infusion). Neither of the serotonin antagonists altered self-administration. In contrast, haloperidol increased reinforced responding, suggesting a shift to the right in the dose/response curve. These data fail to support a role for the serotonin 5-HT3 receptor system in the reinforcing properties of psychostimulants.

537.9

2-ME-5HT-INDUCED DA RELEASE IN THE NUCLEUS ACCUMBENS MEDIATED BY THE 5-HT$_3$ RECEPTORS. L.H. JIANG, L. FISHKIN, S.X. TIAN AND R.Y. WANG. Department of Psychiatry and Behavioral Sciences, SUNY at Stony Brook, Stony Brook, NY 11794-8790.

Using the technique of chronocoulometric recording, we have previous shown that the intraventricular (icv) administration of the 5-HT$_3$ receptor agonist 2-methyl-5HT (2-Me-5HT) dose-dependently increases the DA release in the nucleus accumbens (NAc). The present study was to characterize further the effect produced by 2-Me-5HT. Sprague-Dawley rats were anesthetized with chloral hydrate. A Nafion coated carbon-fiber electrode was calibrated in vitro and then was implanted in the NAc. The ratio for DA/AA and DA/DOPAC obtained from Nafion-coated carbon fiber electrodes was 6015 ± 597 (mean ± SE) and 574 ± 51, respectively, indicating the electrodes were highly selective for detecting DA. Moreover, addition of 5-HT or 2-Me-5HT into the buffer solution did not produce the signal, indicating the DA but not 5-HT is being detected. A computer-aided in vivo electrochemical system (Cypress system) was used to monitor the release of DA. As reported previously, icv injection of 2-Me-5HT dose dependently increased DA release in the NAc. In contrast, icv administration of either saline (n=4), 5-HT$_1$ agonist 5-CT (n=4) or 5-HT$_2$ agonist DOI (n=3) was without effect. 2-Me-5HT-induced increase of DA release could be prevented by icv injection of 5-HT$_3$ receptor antagonist BRL (n=9) and ICS (n=4). However, icv administration of 5-HT$_1$/5-HT$_2$ antagonist metergoline failed to significantly block the electrochemical signal elicited by 2-Me-5HT. These results strongly suggest that the 2-Me-5HT-induced DA release in the NAc is mediated by 5-HT$_3$ receptors in the rat brain. (Supported by USPHS Grants MH-41440 and MH-00378).

537.10

EFFECTS OF THE 5-HT$_3$ RECEPTOR AGONIST 2-METHYL-5HT ON THE NEURONAL ACTIVITY OF A10 DA CELLS. R. Y. WANG AND L.H. JIANG, Department of Psychiatry and Behavioral Sciences, SUNY at Stony Brook, Stony Brook, NY 11794-8790.

We have previously reported that the intraventricular administration of the selective 5-HT$_3$ agonist 2-methyl-5HT (2-Me-5HT) dose dependently increased the DA release in the nucleus accumbens (NAc). Moreover, 2-Me-5HT-induced effect dependent upon the impulse flow of DA neurons. The aim of present study was to determine whether the effects of 2-Me-5HT on the DA release can be explained by its action on the firing rate of A10 DA cells. The techniques of single cell recording and microiontophoresis were used to record A10 DA cells in chloral hydrate anesthetized Sprague-Dawley rats. The concentration of the drugs in the multibarrel electrodes was 10 mM. Of the 88 unidentified A10 DA cells tested with 2-Me-5HT, 33 (37.5%) were excited, 52 (59%) were not affected and 3 were inhibited. Of 12 antidromically-activated A10-NAc DA cells tested, 7 (58%) were activated by 2-Me-5HT. The rest were not affected. Iontophoresis of 2-Me-5HT onto non-DA cells (n=10) in the ventral tegmentum area was without effect. Both 5-HT$_3$ receptor antagonists BRL (n = 10) and ICS (n = 3) but not 5-HT$_1$/5HT$_2$ antagonists ritanserim or metergoline blocked the 2-Me-5HT's excitatory action ($p < 0.01$, t test). In addition, prolonged iontophoresis of high concentration of magnesium, which presumably blocked the neurotransmitter release did not alter the excitatory action of 2-Me-5HT (n = 8), suggesting the effect is a direct one. Our results indicate that 2-Me-5HT induced increase of DA release in the NAc could be accounted for, at least partially, by its excitatory action on the A10-NAc DA neurons. (Supported by USPHS Grants MH-41440, MH-00378).

537.11

CHARACTERIZATION OF 5-HT$_3$ RECEPTORS IN THE RAT MEDIAL PREFRONTAL CORTEX: A MICROIONTOPHORETIC STUDY. C.R. Ashby, Jr., E. Edwards and R.Y. Wang, Dept. of Psychiat. and Behav. Sci., SUNY at Stony Brook, Stony Brook, NY 11794-8790.

We have reported that the iontophoretic application of the 5-HT$_3$ agonist 2-methylserotonin (2-Me-5HT) suppresses the firing of medial prefrontal cortical (mPFc) cells and this effect is blocked by the selective 5-HT$_3$ antagonists granisetron and ICS 205930 (Ashby et al., Eur. J. Pharmacol., 173, 196, 1989). We here report the further characterization of mPFc 5-HT$_3$ receptors in anesthetized male Sprague-Dawley rats, using the techniques of single unit recording and microiontophoresis. The microiontophoresis of 2-Me-5HT and phenylbiguanide (PBG) produced a current-dependent (10-80 nA) suppression of spontaneously active mPFc cells' firing, with the effect of 2-Me-5HT being greater than that of PBG. The iontophoresis of 2-Me-5HT (0.5 - 20 nA) also produced a current dependent suppression of l-glutamate-activated mPFc cells. The microiontophoresis of 1M $MgCl_2$ for 15-20 mins. did not alter 2-Me-5HT's suppressant effect, suggesting that its action is direct. The suppressant action of 2-Me-5HT was blocked by the selective 5-HT$_3$ antagonists granisetron, ICS 205930, LY 278584, MDL 72222, ondansetron and (±)-zacopride at currents of 5-10 nA but not by the antagonists l-sulpiride (D_2), (±)-pindolol (5-HT$_{1A, 1B,}$ ß), mianserin (5-HT$_{1C, 2,}$ H_1), metergoline (5-HT$_{1A, 1B, 1C, 2}$), SCH 23390 (D_1, 5HT$_{1C, 2}$) and SR 95103 ($GABA_A$) The rank order of effectiveness for the 5-HT$_3$ antagonists to block 2-Me-5HT's action is: ICS 205930 = zacopride > granisetron = LY 278584 = ondansetron > MDL 72222. Overall, our results indicate that 2-Me-5HT's suppressant action is directly mediated by 5-HT$_3$ receptors. (Supported by USPHS grants MH-41440, MH-00378 to R.Y.W. and MH-09791 to C.R.A.)

537.12

EFFECTS OF ANTIPSYCHOTIC DRUGS ON THE SUPPRESSANT ACTION OF 2-METHYLSEROTONIN ON MEDIAL PREFRONTAL CORTICAL CELLS: A MICROIONTOPHORETIC STUDY. Y. Minabe, C.R. Ashby, Jr., E. Edwards and R.Y. Wang, Dept. of Psychiat. and Behav. Sci., SUNY at Stony Brook, Stony Brook, NY 11794-8790.

Previously, we have demonstrated that microiontophoresis of the 5-HT$_3$ antagonist 2-methylserotonin (2-Me-5HT) suppresses the firing rate of medial prefrontal cortical (mPFc) cells and this action is blocked by 5-HT$_3$, but not by various 5-HT$_1$/5-HT$_2$ antagonists (Ashby et al., Eur. J. Pharmacol., 173, 196, 1989). Furthermore, we have shown that 2-Me-5HT's action is blocked by the iontophoresis of the atypical antipsychotic drug (APD) clozapine (CLOZ) but not by the typical APDs haloperidol or chlorpromazine (Ashby et al., Eur. J. Pharmacol., 166, 583, 1989). We here report the effects of various putative atypical APDs on the suppressant action of 2-Me-5HT on mPFc cells in anesthetized, male Sprague-Dawley rats. The techniques of single unit recording and microiontophoresis were used. The microiontophoresis of 2-Me-5HT (10-80 nA) produced a current-dependent suppression of mPFc cells firing and this effect was blocked by the atypical APDs CLOZ and RMI 81,852. In contrast, the microiontophoresis (10-20 nA) of the putative atypical APDs CGS 10746B, CL 77328, risperidone, setoperone, sulpiride, SCH 23390 and thioridazine fail to block 2-Me-5HT's action. The intravenous administration of CLOZ, but not CGS 10746B or haloperidol, antagonized the suppressant action of 2-Me-5HT. Our results indicate that among all APDs tested, only CLOZ and RMI 81,852 interact with central 5-HT$_3$ binding sites. Whether the 5-HT$_3$ antagonistic action of these compounds contributes to their therapeutic action or lower potential for inducing neurological side effects remain to be determined. (Supported by USPHS grants MH-41440, MH-00378 to R.Y.W. and MH-09791 to C.R.A.)

537.13

AUTORADIOGRAPHIC LOCALIZATION OF 5HT-3 RECEPTORS IN THE RAT BRAIN USING [^{3}H]-LY278584. D.R. Gehlert, S.L. Gackenheimer*, D.T. Wong and D.W. Robertson. CNS Pharmacology, Lilly Research Labs.,Eli Lilly and Co., Lilly Corporate Center, Indianapolis, IN 46285.

The 5HT-3 receptor appears to mediate the excitatory actions of serotonin in the peripheral nervous system. Initially, it was believed that 5HT-3 receptors were present only in the periphery, but an increasing body of evidence indicates their presence in the brain. In order to localize 5HT-3 receptors in the rat brain, we have utilized the recently introduced high affinity antagonist ligand, [^{3}H]-LY278584.

Twelve micron frozen cryostat sections of rat brain were utilized for a variety of biochemical studies to determine the appropriate conditions for labelling to slide mounted tissue section. Nonspecific binding was determined by the addition of 10 uM 5HT in the incubation buffer. Following these experiments, labeled sections were exposed to tritium sensitive sheet film for 2-6 months.

[^{3}H]-LY278584 bound to tissue sections with a K_d of 0.69 nM and a B_{max} of 79 fmoles/mg tissue and was displaced by 5HT and 5HT-3 specific antagonists. The autoradiographic detection of [^{3}H]-LY278584 binding indicated a widespread distribution of the 5HT-3 receptor in the brain. The highest receptor densities were detected in the area postrema and nucleus of the solitary tract followed by high levels in the substantia gelatinosa of the trigeminal nucleus and spinal cord. Moderate levels of binding were found in the dorsal motor nucleus of the vagus, piriform cortex, olfactory bulb, superficial laminae of the cerebral cortex and the caudal hippocampus. These results indicate that [^{3}H]-LY278584 is a useful tool to study 5HT-3 receptors in the brain by autoradiography.

537.14

EFFECT OF 5HT$_3$ AGONISTS ON *in vitro* ^{3}H-5HT EFFLUX. G. M. Williams, D. L. Smith*, and D. J. Smith. Depts. of Pcol. & Toxicol. and Anes., WVU-HSN, Morgantown, WV 26506.

5HT$_3$ receptors appear to be present on rat spinal synaptosomes (EJP 156:287, 1988), and presynaptic 5HT$_3$ receptors have been shown to modulate neurotransmitter release (TINS 9:424,1986). The current study asks if release-modulating 5HT$_3$ receptors are present on spinal 5HT nerve terminals.

A synaptosomal fraction was isolated from rat spinal cord, incubated with 100nM ^{3}H-5HT, and aliquots superfused at 37°C with TRIS-buffered Krebs solution. A pre-drug fraction and a during-drug fraction were collected from each superfusion chamber.

2CH$_3$5HT and phenylbiguanide (PBG) did not alter basal efflux at concentrations < 1 uM. At concentrations ≥ 100 nM bufotenine increased basal efflux of both ^{3}H-5HT and ^{3}H-5HIAA. This pattern suggests that the agent may disrupt ^{3}H-5HT storage rather than promote release via receptors on the terminal surface. 5HT, PBG and 2CH$_3$5HT were also tested for effects on evoked release. At concentrations ≤ 1uM, only 5HT altered 15mM K^+-induced ^{3}H release, and 1 uM ICS 205-930 did not block this effect.

It would appear that 5HT$_3$ receptors are not involved in the regulation of synaptosomal ^{3}H-5HT efflux in the rat spinal cord tissue.

SECOND MESSENGERS VI

538.1

INDEPENDENT EFFECTS OF pH_i, Ca^{2+} AND PHOSPHODIESTERASE ON THE cAMP-GATED Na^+ CURRENT IN NEURONS OF THE MOLLUSK *Pleurobranchaea*. V. Mazzarella and R. Gillette, Neuroscience Program and Dept. of Physiol. & Biophys., University of Illinois, Urbana, IL 61801. Na^+ current gated by cAMP ($I_{Na,cAMP}$) in the ventral white cell buccal neurons of *Pleurobranchaea* is sensitive to small changes in intracellular pH (pHi), intracellular Ca^{2+}, and phosphodiesterase activity (PDE). We examined interactions of pH_i, Ca^{2+}, and PDE in voltage clamp experiments measuring the $I_{Na,cAMP}$ response to injected cAMP. Inhibition of PDE by IBMX causes increased current amplitude and slowed decay of the current. Intracellular acidification or alkalinization by addition of $NaHCO_3$ or $(NH_4)_2SO_4$ to the bath increases the amplitude but not the decay rate of the current. The constant decay rate in these experiments suggests that a mechanism other than a pH effect on PDE causes the increase in current amplitude. $I_{Na,cAMP}$ is suppressed by intracellular Ca^{2+} (J. Neurophys. 59:248). In the VWC, Ca^{2+} chelation by pressure injection of BAPTA into the cell did not alter the decay of $I_{Na,cAMP}$, suggesting that the inhibitory effect of calcium is not mediated via the PDE. Furthermore, the chelation of calcium did not diminish the increase in amplitude caused by intracellular acidification or alkalinization. Removal of extracellular calcium increased $I_{Na,cAMP}$ amplitude but not decay rate. When pH_i was decreased under these conditions, a further increase in amplitude is observed, indicating different modulation mechanisms for calcium and pH.

538.2

ALTERATIONS OF Na^+ DIFFERENTIALLY AFFECT AGONIST-INDUCED PHOSPHOINOSITIDE HYDROLYSIS IN RAT BRAIN SLICES. G.C. Ormandy, X. Li, L. Song*, M.B. Williams and R.S. Jope. Dept. of Psychiatry, University of Alabama at Birmingham, Birmingham, AL 35294.

The hydrolysis of inositol-containing phospholipids is an important signal transduction mechanism in the brain. Phosphoinositide hydrolysis in vitro is known to be influenced by the concentration of both K^+ and Ca^{2+} ions. In this study using rat brain slices we measured the effect of altered Na^+ concentrations on phosphoinositide hydrolysis induced in response to a number of agonists. Reductions of the Na^+ concentrations below 120 mM resulted in incremental increases in basal and norepinephrine stimulated accumulation of ^{3}H-inositol monophosphate in cortical slices that had been prelabelled with ^{3}H-inositol, and maximal responses were obtained with 0 and 5 mM Na^+. Similar effects of Na^+ were also observed in hippocampal and striatal slices. In contrast the responses to carbachol and ibotenate were similar in medium containing 120 or 5 mM Na^+. These results demonstrate that alterations of Na^+ concentration may differentially influence the effect of agonists on phosphoinositide hydrolysis.

538.3

SODIUM MODULATES CYCLIC AMP PRODUCTION IN RAT BRAIN SLICES. X. Li, L. Song* and R.S. Jope, Departments of Psychiatry and Pharmacology, University of Alabama at Birmingham, Birmingham, AL 35294.

Reduction of the sodium concentration from 120 mM to 5 mM in the incubation media dramatically increased the basal cyclic AMP concentration in rat brain slices, with or without rolipram to inhibit phosphodiesterase. This effect of low sodium was not changed by altering the calcium concentration or adding EGTA in the incubation media. Isoproteronal and norepinephrine (NE) significantly stimulated cyclic AMP production in slices from rat brain cortex, hippocampus and striatum. Reduction of sodium did not change isoproterenol-stimulated cyclic AMP, but significantly enhanced NE-stimulated cyclic AMP production. Ibotenate, an excitatory amino acid agonist, stimulated cyclic AMP production to twice the basal concentration, and this effect was not altered by lowering the sodium concentration in the media. In normal sodium, low concentrations (1 μM to 100 μM) of quisqualate slightly increased cyclic AMP, but 1 mM quisqualate significantly inhibited basal and Isoproteronal - or NE-stimulated cyclic AMP. However, in low sodium, the stimulatory effect of quisqualate was significantly increased at 1 μM to 100 μM, and the inhibitory effect was reduced by 50% or more.

These results suggest that sodium modulates cyclic AMP production, possibly by more than one mechanism. Phosphodiesterase inhibition is not likely to be involved and the effect is calcium-independent. Potentiation of NE-stimulated cyclic AMP by low sodium may be indirectly mediated by activating PI hydrolysis. The stimulatory effect of excitatory amino acids is independent of sodium concentration, whereas blockade of quisqualate-inhibited cyclic AMP production indicates a regulatory effect of sodium in neuronal activity.

538.4

MAITOTOXIN CAUSES INCREASED cAMP LEVELS IN SK-N-SH NEUROBLASTOMA CELLS. Jesse Baumgold and Robert Paek*, Dept. of Radiology, George Washington Univ. School Med., Washington, DC 20037.

We have previously reported that stimulation of muscarinic receptors in SK-N-SH cells causes increased intracellular cAMP via a pathway that is independent of protein kinase C (BBRC 154 1137, 1988) and that may involve stimulation of a calcium-calmodulin dependent adenylate cyclase (Soc. Neurosc. Abstract, 1988). The goal of the current work was to induce a similar increase in cAMP by stimulating other PI turnover-coupled receptors. Although SK-N-SH cells express bradykinin and histamine receptors, stimulation of these receptors produced only a fraction of the PI response obtained by carbachol (17% and 21%, respectively). Neither response was sufficient to increase cAMP levels. Maitotoxin has been shown to stimulate PI turnover directly and to increase intracellular calcium in a variety of neuronal cells (Mol.Pharm. 36 44, 1989). In SK-N-SH cells, 0.5 ng/ml of maitotoxin caused increasd PI turnover and an increase in cAMP levels. These results demonstrate that direct stimulation of PI turnover by maitotoxin also increases cAMP formation and further substantiates the hypothesis that stimulation of PI turnover can increase cAMP levels by stimulating a calcium/calmodulin dependent adenylate cyclase.

538.5

ATTENUATION OF cAMP EFFLUX BY COPPER: A MECHANISM FOR AMPLIFICATION OF PGE_2 ACTION IN THE BRAIN. P.Magni*, M.Sanghera and A.Barnea. Depts. Obstet. Gynecol., Physiol. & Psych., Univ. Texas Southwest.Med.Ctr., Dallas, Tx 75235

It is known that PGE_2 stimulation of peptide release (LHRH) from the median eminence (ME) is mediated by the cAMP pathway, and a short pre-treatment with copper (Cu) markedly amplifies this release process. Since in many tissues stimulation of cAMP accumulation is accompanied by cAMP efflux, we considered the possibility that Cu-amplified PGE_2 action is a result of attenuation of cAMP efflux. When rat ME were incubated in vitro, PGE_2 induced a rapid (<2.5 min) and sustained (15 min) cAMP efflux, the degree of which was a function of [PGE_2]: by 5 min exposure to 10 μM PGE_2, efflux was 584% of basal (100%) and it accounted for 12% of the total (tissue + medium) cAMP. A 5 min pre-treatment with Cu inhibited (49-66%) PGE_2-induced efflux regardless of the length of PGE_2-exposure and [PGE_2]; this reduced level of cAMP was not due to phosphodiesterase activity in the incubation medium. Unlike the dramatic increase in cAMP efflux, PGE_2 induced a moderate 37% increase in content which was not altered by Cu. Thus, the size of the functional pool of cAMP is very small relative to the total cellular cAMP and hence, changes in stimulated cAMP-efflux are not reflected in the tissue content. Since Cu amplifies PGE_2 stimulation of LHRH release, these results are consistent with Cu attenuation of cAMP efflux as a mechanism for amplification of PGE_2 action mediated by the intracellular cAMP pathway.

538.6

EFFECTS OF FORKOLIN ON DOPAMINE AND ACETYLCHOLINE RELEASE IN RAT NEOSTRIATAL SLICES. H. Lee*, V. Reid*, and M.H. Weiler. Univ. of Wisconsin, School of Pharmacy, Madison, WI 53706.

The involvement of the cyclic AMP (cAMP) effector system in the release of dopamine (DA) and acetylcholine (ACh) from rat neostriatal slices was assessed. Forskolin, an activator of adenylate cyclase, was used to enhance cAMP levels, and the consequence of this enhancement on potassium-stimulated release of DA and ACh from the same slice preparation was evaluated. Forskolin increased K^+-stimulated DA release in a dose-dependent manner. In the presence of 1, 10 and 50 μM forskolin, DA release increased 2- (p=0.15), 3- (p=0.0013) and 4.5-fold (p=0.000), respectively. ACh release from the same slices from which DA release was monitored was not altered in the presence of forskolin. This increase in DA release but no change in ACh release by forskolin occurred when acetylcholinesterase activity was either inhibited or intact (choline release was monitored). It was also confirmed that forskolin induced a dose-dependent increase in cAMP in the slices under similar experimental conditions. That forskolin increases neostriatal cAMP levels and enhances DA release but has negligible effects on ACh release suggests that the cAMP effector system is involved in mechanisms that increase DA release but not in mechanisms that enhance ACh release. Supported by grants from NIH (AG05953) and the American Parkinson's Disease Association.

538.7

A COMPARISON OF THE REGULATORY PROPERTIES OF STRIATAL AND CORTICAL FORMS OF ADENYLATE CYCLASE. L.A. DOKAS AND S.M. TING*. Departments of Neurology and Biochemistry, Medical College of Ohio, Toledo, OH 43699.

This study was undertaken to compare the properties of rat striatal and cortical forms of adenylate cyclase. Enzyme activity was measured in the lysed mitochondrial fraction with a radioisotope assay as described by Gil and Wolfe (JPET 232: 608, 1985). Although basal enzyme activity (approx. 400 pmol cyclic AMP/min/mg protein) is the same in both preparations, the striatal form is more responsive to forskolin (8-fold stimulation at 10^{-5} M) than is the cortical form (4-fold stimulation). Conversely, cortical adenylate cyclase activity is stimulated more by GTP. In both cases, 10^{-4} M GTP limits maximal forskolin stimulation to 60% of that seen with forskolin alone. Cholinergic agonists inhibit adenylate cyclase activity in both brain regions in the order oxotremorine > acetylcholine ≥ carbachol. More inhibition is seen in response to acetylcholine in the cortex. Although acetylcholine inhibits the striatal enzyme equally ± forskolin, less inhibition is seen with the cortical form in the presence of forskolin. The general muscarinic antagonist atropine blocks the effect of 10^{-3} M acetylcholine in both striatum and cortex, while the more selective antagonist pirenzepine has little effect at concentrations up to 10^{-5} M, suggesting a high-affinity, pirenzepine-insensitive receptor (m2 or m4) is involved. [D-Trp^8]-somatostatin inhibits both forms equally. These results indicate significant differences in interactions among subcomponents of the adenylate cyclase complex in striatal and cortical membranes. Supported by grants from NIH (NS 23598) and the Ohio Department of Aging.

538.8

DIHYDREXIDINE IS A POTENT AND FULLY EFFICACIOUS D_1 DOPAMINE RECEPTOR AGONIST IN VIVO AND IN VITRO. **L.L. Cook, D.M. Mottola, W.K. Brewster, D.E. Nichols, M.H. Lewis, R.B. Mailman. Brain and Development Research Center, Univ. of North Carolina, Chapel Hill, NC 27599 and Purdue Univ., West Lafayette IN 47907.**

Dihydrexidine (DHBP) is a new D_1 dopamine receptor agonist which has been shown, using in vitro studies, to be potent and fully efficacious relative to dopamine. This work characterizes the effects of DHBP on stimulation of D_1 receptors in vivo (i.e., using microdialysis to sample cellular cAMP efflux) and in vitro (i.e., using superfused striatal slices). For microdialysis studies, 4 mm unilateral probes were inserted in the striatum of conscious adult male rats via guide cannulae stereotaxically implanted 3–5 days previously. The probes were perfused overnight with Krebs Ringer buffer (2 μL/min). Samples were collected every 30 min, and cAMP quantified by RIA. Phosphodiesterase inhibitors like IBMX or rolipram were included to permit measurement of both basal and stimulated levels of cAMP efflux. Perfusion with 1 mM rolipram resulted in basal cAMP efflux of 0.48 ± 0.15 fmole/μL (n=5). DHBP at concentrations from 30–1000 μM produced a dose–dependent increase in cAMP, to a maximal increase of 300% of basal levels.

Similarly, superfused striatal slices were used to study DHBP–stimulated cAMP efflux. Slices were prepared with a McIlwain tissue chopper, and perfused (0.1 mL/min and 37°C) with oxygenated buffer containing 1 mM IBMX with or without test drugs. Fifteen min samples were collected for measurement of cAMP by RIA. DHBP was at least ten–fold more potent than DA and was fully efficacious in stimulating cAMP efflux from superfused striatal slices. The possible inhibitory effects of DHBP on uptake of DA and 5–HT were assessed to determine if these effects might be attributable to endogenous dopamine. Uptake of ^{3}H–DA and ^{3}H–5–HT was measured in striatal and cortical synaptosomes, respectively. Relative to its receptor potency (Lovenberg et al. Eur. J. Pharmacol. 166:111, 1989), DHBP was essentially 10^3 less active at either DA (EC50 = 50.7 μM) or 5–HT (EC50 = 23.3 μM) uptake sites. Together, these data demonstrate that dihydrexidine is a potent and fully efficacious D_1 agonist in two functionally intact preparations, and that these neurochemical effects are consistent with direct (rather than indirect) D_1 receptor action. (Supported by ES07126, ES01104, MH40537, and MH42705).

538.9

CANNABINOIDS AND AMINOALKYLINDOLES BIND TO COMMON RECEPTORS TO INHIBIT ADENYLYL CYCLASE IN BRAIN. S. R. Childers, M.A. Pacheco, and S.J. Ward, Dept. Physiol. and Pharmacol., Bowman Gray Sch. Med., Winston-Salem, NC 27103, and Sterling Research Group, Sterling Drug Inc., Rensselaer, NY 12144.

Both cannabinoids (Cn) and aminoalkylindoles (AAI) bind to specific G-protein-coupled membrane receptors as demonstrated by radioreceptor binding, inhibition of isolated smooth muscle contractions and GTP-dependent inhibition of adenylyl cyclase in rat cerebellar membranes. Receptor binding assays have demonstrated that Cn and AAI bind to common receptors. The current experiments support this conclusion by examining effects of both Cn and AAI on adenylyl cyclase (AC) in brain. In rat cerebellar membranes, both AAI agonists and the potent Cn levonantradol inhibited AC with the same efficacy. The IC_{50} values for levonantradol and the most potent AAI agonists in inhibiting AC were 0.1-0.3 μM. The dose response curves of both Cn- and AAI-inhibited AC were shifted to the right by an AAI antagonist. The regional distributions of Cn- and AAI-inhibited AC in rat brain were identical, with maximal inhibition occurring in striatum and cerebellum. In cerebellum, no additivity in inhibition occurred when maximally inhibitory concentrations of both Cn and AAI agonists were added in the same assay tubes. Both AAI agonists and Cn inhibited cyclic AMP levels in intact cultured rat cerebellar granule cells to the same extent, and these inhibitory actions were blocked by the AAI antagonists. Also, AAI-inhibited cyclic AMP levels were blocked by treatment of cells with pertussis toxin. These data suggest that Cn and AAI bind to common G-protein-linked receptors which inhibit AC in rat brain membranes and in cerebellar granule cells.

538.10

S-ADENOSYL-L-METHIONINE MODULATES RECEPTOR-MEDIATED cAMP PRODUCTION IN RAT BRAIN. P. Zhong, J. McWilliam* and K.J. Kellar. Dept. of Pharmacology, Georgetown Univ. Med. Center, Wash., DC 20007.

S-adenosyl-l-methionine (SAM) can increase receptor-mediated cAMP production in several tissues in vitro. The mechanism for this effect is not known, but both phospholipid methylation (Hirata and Axelrod, Science **209**:1082, 1980) and protein carboxyl methylation (Backlund and Aksamit, J. Biol. Chem., **263**:15864, 1988) by SAM have been proposed as important steps in signal transduction. We investigated the effects of SAM on receptor-mediated stimulation and inhibition of cAMP production in brain slices from male, Sprague-Dawley rats (450 g). Slices were washed and preincubated with SAM and 50 μM IBMX before stimulation of cAMP production under the same incubation conditions. SAM potentiated cAMP production by norepinephrine (NE), 6F-NE and isoproterenol with an EC_{50} of = 60 μM. SAM also attenuated the inhibitory effect of the 5-HT-1A agonist DPAT on forskolin stimulated cAMP production. Neither the basal levels nor forskolin stimulated cAMP was affected by SAM. A single i.p. injection of SAM (20 mg/kg) 30 min before sacrifice potentiated cAMP production by NE in vitro. Treatment of rats for 2 weeks (10 mg/kg, bid) did not affect NE-stimulated cAMP production nor did it affect α-1 or ß-adrenoceptor nor 5-HT-2 receptor binding. However after the chronic treatment, SAM in vitro no longer potentiated NE-stimulated cAMP production. In addition, the chronic treatment with SAM appeared to blunt the inhibition of forskolin stimulated cAMP by DPAT. These data suggest that SAM can have potentially important modulatory effects on signal transduction mechanisms. (Supported by grant #MH 41819)

538.11

MELATONIN ACTS VIA TWO G-PROTEINS TO INHIBIT ADENYLATE CYCLASE. PJ Morgan and P Barrett*. Rowett Research Institute, Aberdeen, Scotland, UK, AB2 9SB

Melatonin receptors have been localized on the cells of the mammalian pars tuberalis (PT), and have been shown to be coupled through inhibitory G-proteins to adenylate cyclase (Morgan *et al.*, J. Mol. Endocrinol. 3:R5, 1989). The inhibitory effect of melatonin on intracellular levels of cyclic AMP could only be seen after stimulation with forskolin. *Bordetella pertussis* toxin (IAP) has been used to investigate the nature of the G-protein coupling between the melatonin receptor and adenylate cyclase.

Primary cultures of ovine PT cells were exposed to different doses of IAP (0.5-500 ng/ml) for 16 h, and the effect on the inhibition of forskolin-stimulated cyclic AMP formation by melatonin measured, as described previously (Morgan *et al.*, 1989). IAP attenuated melatonin's inhibitory effect at 10 ng/ml reaching a maximal effect by 100 ng/ml, yet even at this concentration and higher the melatonin response was not completely abolished, with significant ($p<0.001$) inhibition (>50%) still attainable. This result indicates that both an IAP-sensitive and IAP-insensitive G-protein are linked to the melatonin receptor.

Using ^{32}P-NAD, IAP (20 μg/ml) was shown to catalyze the ADP-ribosylation of a 41 kd protein in membranes, separated by 12.5% SDS-PAGE, confirming the presence of IAP-sensitive G-protein (G_i) in the PT. Furthermore in membranes prepared from PT cells pre-treated with IAP (0.5 ng/ml), the subsequent radiolabelling with ^{32}P-NAD was reduced.

The binding of 2-^{125}I-iodomelatonin (I-MEL) to PT membranes is regulated through G-proteins. IAP (0.1-20 μg/ml) attenuates I-MEL binding to PT membranes in a dose-dependent manner, reaching a maximum effect of 20% inhibition. GTP (1 mM) also attenuates binding by 40%, and in combination with IAP this effect is additive. Both of these changes occur through G-protein regulated alteration in melatonin receptor affinity, but IAP alters this affinity through covalent modification of a G-protein, whereas GTP acts via a reversible binding reaction. As IAP only inhibits 20% of I-MEL binding, this indicates that not all G-proteins linked to the melatonin receptor are IAP-sensitive G_i. This is emphasized by the fact that GTP can still inhibit I-MEL binding further after IAP treatment. These results confirm that both an IAP-sensitive and an IAP-insensitive G-protein must link the melatonin receptor to adenylate cyclase.

538.12

LOCALIZATION OF ADENYLATE CYCLASE AND GLUCOSE TRANSPORTER IN RAT BRAIN USING [^{125}I]-LABELED DERIVATIVES OF FORSKOLIN. N.M. Appel, K.B. Seamon* A. Laurenza*, I.A. Simpson* and E.B. De Souza. NIDA/ARC, Baltimore, MD 21224; FDA and NIDDK, Bethesda, MD 20892.

Two iodinated derivatives of forskolin have been synthesized that show differential specificity for adenylate cyclase (6-[^{125}I]Fsk, 2200 Ci/mmol) and for the glucose transporter (7-[^{125}I]Fsk, 2200 Ci/mmol). These iodinated compounds have been used to localize forskolin binding sites in rat brain sections using autoradiography. The distribution of both 6-[^{125}I]Fsk and 7-[^{125}I]Fsk binding sites was similar to that previously reported for [^{3}H]forskolin. Highest densities were noted in caudate putamen, nucleus accumbens, olfactory tubercle, substantia nigra (reticularis), superior colliculus (superficial grey) and cerebellar cortex (molecular layer). Binding in cerebral cortex and hippocampus was less intense, but while binding in cerebral cortex was uniform, binding in hippocampus was enriched in the molecular layer of dentate gyrus and pyramidal cell layer of regions CA2 and CA3. The binding of 6-[^{125}I]Fsk was inhibited by forskolin but not by 1,9-dideoxyforskolin, consistent with these sites being associated with adenylate cyclase. Agents that inhibit forskolin binding to the glucose transporter, such as cytochalasin-B and D-glucose, decreased 7-[^{125}I]Fsk binding but did not decrease 6-[^{125}I]Fsk labeling. Thus it appears that 6-[^{125}I]Fsk binds exclusively to adenylate cyclase and can be used as a specific ligand to measure adenylate cyclase binding sites. In contrast, 7-[^{125}I]Fsk binds to sites that are associated with the glucose transporter binding sites in brain. These novel derivatives can be used to study the differential localization of enzymes involved in signal transduction and proteins involved in glucose utilization.

538.13

CHARACTERIZATION OF D_1-STIMULATION AND D_2-INHIBITION OF ADENYLATE CYCLASE ACTIVITY IN RAT REGIONAL BRAIN AREAS. E.B.Hollingsworth and B.G.Huff*, Div. of Pharma., Burroughs Wellcome Co., Res. Tri. Pk., NC 27709.

Forskolin-induced, activation of adenylate cylase was used to explore dopamine$_1$ (D_1) stimulation and dopamine$_2$ (D_2) inhibition of adenylate cyclase accumulation in rat brain slices from striatal and limbic regions. Stimulation of cyclic AMP production was measured by quantitating the conversion of [3H]-adenine to [3H]-cyclic AMP. In striatal and limbic regions, the EC_{50} value for SKF38393-induced D_1-stimulation of adenylate cyclase, 3 x 10^{-9}M, was comparable to published values from other methodologies. The selective D_1 antagonist SCH23390 inhibited this stimulation. The D_2 agonist, PPHT, at 1 x 10^{-9}M, inhibited the D_1-stimulated increase in cyclic AMP accumulation in the striatal region by 50%. The D_2 antagonist, eticlopride, inhibited this accumulation, which indicates that the inhibition of adenylate cyclase by PPHT is a D_2 receptor-specific event. The D_1-mediated increase in cyclic AMP accumulation was detected in the brain areas that have the highest density of D_1 receptors. The D_2-mediated inhibition of cyclic AMP accumulation was seen in the striatal but not in the limbic region. The assay system characterized here enables efficient study of D_1-and D_2-receptor interaction and modulation.

538.14

COUPLING OF AMYGDALOID NEUROPEPTIDE RECEPTORS WITH THE ADENYLATE CYCLASE SECOND MESSENGER SYSTEM. S. Eldon,* R. Scibilia, C. Kilts. Duke Univ. Med. Ctr., Durham, N.C. 27710.

The amygdaloid complex is remarkable in the density and diversity of neuropeptides and neuropeptide receptors. We examined the effect of selected neuropeptides on the efflux of cyclic AMP from parallelepiped slices of the rat amygdaloid complex as an estimate of receptor effector coupling. Vasoactive intestinal peptide (VIP) produced a concentration-dependent increase in cyclic AMP efflux which did not vary between amygdaloid nuclei differing in VIP binding site density. Neither corticotropin releasing factor (CRF) nor neurotensin (0.1-30 uM) affected cyclic AMP efflux. However, neurotensin inhibited forskolin (10 uM)-stimulated cyclic AMP efflux. The delta opioid receptor agonist [D-Pen-D-Pen] enkephalin produced a significant inhibition of forskolin-stimulated cyclic AMP efflux. The inhibitory effect of the mu opioid receptor agonist (DAGO) was of lesser magnitude and similar to that of somatostatin. These results indicate that some amygdaloid neuropeptide receptors are positively and negatively coupled to adenylate cyclase and that receptor-effector coupling does not covary with receptor density. (MH-39967)

538.15

HORNET VENOM SAC EXTRACT ELEVATES INTRACELLULAR CYCLIC AMP IN HUMAN CILIARY EPITHELIAL CELLS A.R. Heath, J.S.Ishay*[1] and D.E.Potter*. Baylor College of Medicine, Center for Biotechnology, The Woodlands, TX 77381, USA and [1]Sackler Faculty of Medicine, Tel-Aviv University, Israel.

The neuroepithelial cells of the human ciliary body play a major role in the control of intraocular pressure (IOP) through their involvement in ion flux and aqueous humor formation. They possess several G protein-mediated and adenylate cyclase-linked receptors that have also been implicated in IOP regulation. Complete extracts of venom sacs (VSE) from the Oriental Hornet, Vespa orientalis, which contain several biogenic amines and peptides, have been shown to lower IOP radically (Kam, J et. al., *Comp. Biochem. Physiol.*, 92C:329, 1989). To determine the site and mechanism of action of VSE, we examined its effect on intracellular cyclic AMP (cAMP) production by human, transformed, non-pigmented ciliary epithelial cells (NPCEC) in culture (ODM Cl-2 line, Martin-Vasallo, P et. al., *J. Cell. Physiol.*, 141:243, 1989). VSE (a homogenate of one venom sac/0.4 ml Earle's BSS) induced a dose-related increase in intracellular cAMP as measured by RIA. Cultured NPCEC retain the native responsiveness of ciliary epithelial cells to beta-adrenergic agonists. Isoproterenol increased cAMP in a dose-related fashion (10^{-9} M to 10^{-4} M) with an average increase over basal levels of 66 ±12-fold at 10^{-5} M (n=8). Propranolol blocked this response but did not suppress the response to VSE. Thus, elevated cAMP levels in NPCEC following VSE challenge do not result from activation of the beta-adrenoceptor but may be due to other amines or peptides. Work is in progress to address this question. (Supported by EY06338).

538.16

ROLE OF TUBULIN IN THE REGULATION OF ADENYLYL CYCLASE IN C6 CELLS K. Yan, N. Wang, A. Ringer and M. M. Rasenick, Physiology &Biophysics, and Committee on Neuroscience, University Illinois College of Medicine, Chicago, IL 60680

Tubulin polymerized with GTP or hydrolysis resistant GTP analogs stimulates adenylyl cyclase activity in C6 membranes, probably via direct guanine nucleotide transfer from tubulin to Gsα. The modulation of adenylyl cyclase activity by tubulin appears to be agonist independent in the tightly coupled permeable C6 cell and membranes. Tubulin is able to stimulate the adenylyl cyclase even in the presence of l-propanolol, and in isoproterenol-desensitized C6 cells, where free GppNHp is without effect. We have also showed that there is a direct interaction between tubulin and G-proteins by using hybridization techniques. A high selectivity of tubulin binding to αs and αi1 has been found. It is known that there is a significant amount of tubulin in the plasma membranes (about 6% of the total membrane proteins), however, the role of this protein in the membrane remains largely unknown. In gel overlay experiments, [^{32}P]-AAGTP-transducin binds to both microtubule tubulin and membrane tubulin. Two classes of membrane tubulin can be distinguished on the basis of the hydrophobicity; αs associates with the more hydrophobic class whereas αi binds better to the less hydrophobic class. Proteolysis experiments indicate that the "binding site" on tubulin for G-proteins is near the carboxyterminal. Efforts are underway to determine the interaction between the membrane and these domains of tubulin as they relate to modulation of the signal transduction process.

538.17

TUBULIN-G PROTEIN INTERACTION: THE DYNAMIC ROLE OF THE CYTOSKELETON IN REGULATION OF THE NEURONAL SIGNAL TRANSDUCTION. N. Wang, K. Yan and M.M. Rasenick. Dept. of Physiology and Committee on Neuroscience, Univ. of Illinois at Chicago, Chicago, IL. 60680.

Tubulin, a GTP-binding protein with structural similarities to signal-transducing G proteins, has been implicated as a modulator of the neuronal adenylyl cyclase. This laboratory has previously shown that tubulin with GTP or hydrolysis-resistant GTP analogs bound is capable of inhibiting adenylyl cyclase. Photoaffinity labeling studies suggest a direct transfer of nucleotide from tubulin to the inhibitory GTP-binding protein, Gi. Hybridization as well as immunoprecipitation studies show a direct interaction between tubulin and α subunits of G proteins. This interaction is specific and distinct for different subtypes of Gα (J. Biol. Chem. 265:1239, 1990). Further studies designed to determine the Gα-binding domains of tubulin reveal that despite of the effectiveness of tubulin dimers, tubulin polymers (microtubules or rings) are inefficient in inhibiting adenylyl cyclase and binding to Gα. Furthermore, the polymerization of tubulin was inhibited by G proteins, implying that the tubulin-Gα interaction may involve tubulin domains which are located in or close to the tubulin domains involved in tubulin polymerization. These data suggest that tubulin, usually considered to function as a structural component in fully-differentiated neuronal cells may play a dynamic role in regulation of neurotransmitter response or responsiveness.

538.18

EFFECTS OF TUBULIN ON AGONIST BINDING AFFINITY IN C6 GLIOMA CELLS. M. Watanabe and M. M Rasenick, Dept. of Physiology and Biophysics and Committee on Neuroscience, University of Illinois at Chicago, Chicago, IL 60608

Guanine nucleotides have been known to modulate agonist binding affinity for G protein linked receptors. Our laboratory demonstrated that tubulin, which is an element of the cytoskelton, alters the coupling between receptors and G proteins involved in the stimulation or inhibition of adenylate cyclase via direct transfer of GTP from tubulin to Gsα and Gi1α. However, since results from many laboratories suggest that G protein modulation of receptor affinity is a process quite different from G protein modulation of adenylate cyclase, it was not clear that added tubulin would result in change in receptor affinity.

In this study, the effects of tubulin-GppNHp have been compared to those of GppNHp in changing β-adrenergic agonist affinity in both permeable C6 cells (by a newly devised method) and C6 membranes. Competitive binding studies were performed with isoproterenol and the β-adrenergic antagonist, iodopindolol. Tubulin-GppNHp was more effective than GppNHp in decreasing receptor affinity in both cells and membranes. These results suggest that tubulin might modulate neuronal signal transduction at multiple loci.

538.19

MODULATION OF ADENYLYL CYCLASE ACTIVITY BY G PROTEIN SPECIFIC SYNTHETIC PEPTIDES IN C6 GLIOMA CELLS. M.B.Lazarevic, M.M.Rasenick & H.Hamm. Department of Physiology & Biophysics and the Committee on Neuroscience, University of Illinois College of Medicine, Chicago, IL 60680.

Coupling of a neurotransmitter to a G protein and the subsequent activation of an intracellular effector (e.g. adenylyl cyclase, ion channels) by that G protein are processes which have been the subject of considerable experimentation over the past two decades. Despite this, little is known about the molecular details of receptor- G protein interaction in situ, especially as it relates to the G protein. We chose to explore β adrenoceptor-effector coupling in C6 glioma cells by introducing peptides which correspond to various domains of the G proteins, αs and αi2. These peptides were introduced into saponin-permeable C6 cells (which show a "tight coupling" between β adrenoceptor and Gs) as well as C6 membrane (in which this coupling is much lower) and effects upon adenylyl cyclase activity were measured. Peptides corresponding to the C terminal part of αs decreased isoproterenol stimulation of adenylyl cyclase in a dose dependent fashion. Curiously, in C6 membranes, adenylyl cyclase activation by GppNHp (receptor-independent) was blocked by these peptides as well. Furthermore, peptides corresponding to a similar region on αi2 increased adenylyl cyclase activity in either system. It is possible that this supports the idea of some interaction between the αi and αs subunits. These results demonstrate that synthetic peptides are a useful tool for the exploration of receptor-G protein coupling.

539.1

CHANGES IN DOPAMINE (DA) MEDIATED BEHAVIORS AND IN STRIATAL D2 DOPAMINE RECEPTOR (DAr) BINDING FOLLOWING NEUROTOXIC DOSES OF AMPHETAMINE. JZ Fields, LJ Wichlinski, K Engh, J Cronborg & JH Gordon. Res Svce 151, VA Hosp, Hines, IL 60141 and Dept of Pharmacology, Loyola Univ Med Sch, Maywood, IL 60153.

Acute injection of a single dose of amphetamine (A) (9.2 mg/kg, IP) in combination with iprindole (I) (10.0 mg/kg, IP) is known to produce, similar to repeated high doses of A or meth-A, a long-lasting depletion of DA in certain brain areas, particularly, striatum. Last year (SN Abst. #519.7, 1989, L. Wichlinski et al) we reported increases in apomorphine (APO) induced behaviors in male rats (locomotion, stereotypy) 3 & 10 weeks following A/I. We now report [1] replication of these behavioral data; [2] confirmation of DA depletion (at 4 weeks) in striatum (-49%) but not n. accumbens(-6%); [3] increases (+53%, 12 weeks post-A/I) in Bmax (but not Kd) for sulpiride sensitive [3H]spiroperidol binding to striatal D2 DAr ($p < .05$). Furthermore, the peptide cyclo(leu-gly)(CLG), a neuromodulator which we showed down-regulates DA receptors in many different paradigms, significantly reduced APO-induced behaviors in A/I rats and reversed the increases in D2 DAr binding (fmol/mg prot: con=17.4; A/I=26.6; A/I + CLG=21.6, $p < .05$). A/I may represent a useful model of long-term D2 DAr up-regulation. (Supported by grants from the VA, Scottish Rite Schizophrenia Res Pgm-NMJ, & NIH [NS26449]).

539.2

INCREASED SENSITIVITY TO GTP OF STRIATAL ADENYLATE CYCLASE ACTIVITY INDUCED BY PROLONGED ADMINISTRATION OF D1 AND D2 RECEPTOR ANTAGONISTS. C. Ventra, T. Florio, M. Grimaldi*, E. Landolfi*, and G. Schettini. Dept. of Pharmacology, II School of Medicine, Univ. of Naples, via S. Pansini 5, 80131 Naples, Italy.

Long term administration of neuroleptics is frequently associated with the development of receptor supersensitivity. We studied the response to GTP of striatal adenylate cyclase activity in membranes from D1, D2 and D1 + D2 supersensitive rats to evaluate the role of G-proteins in the coupling of rat striatal dopamine receptors to adenylate cyclase activity of up-regulated receptors. SCH-23390, Haloperidol, SCH-23390 + Haloperidol and saline was administered to male Wistar rats over a 21 days period. Our results show that, besides the expected variation of dopamine and D1 agonist (SKF-38393) stimulated adenylate cyclase activity induced by up-regulated receptors, also the response of the enzyme activity to GTP alone is modified. These results suggest that the increased sensitivity of G-proteins to GTP may participate in the mechanisms of induction of receptor up-regulation and be involved in their enhanced functional response.

539.3

EFFECTS OF CHRONIC TREATMENT WITH LOW DOSES OF 1-SULPIRIDE ON DOPAMINE RECEPTOR AND B-ADRENOCEPTOR FUNCTION IN RAT STRIATUM AND FRONTAL CORTEX. C. Missale, S. Sigala*, P. Rizzonelli*, A. Forgione* and P.F. Spano. Inst of Pharmacol Exp Ther, Sch of Med, Univ of Brescia, Italy.

There is now increasing evidence that 1-sulpiride has antidepressant activities when administered at low doses. Down regulation of ß-adrenoceptor function in the frontal cortex is a well documented adaptive response to chronic administration of antidepressants. On these basis we studied the responses of striatal and cortical DA receptors and ß-adrenoceptors to chronic administration of low doses of 1-sulpiride. Male Sprague-Dawley rats were treated with 2 mg/kg (i. p.) 1-sulpiride twice a day for 21 days and killed by decapitation 8 days after the last injection. The results showed that the function of striatal D-1 and D-2 receptors is decreased by 1-sulpiride administration, suggesting that at low doses 1-sulpiride preferentially blocks DA autoreceptors controlling DA release. 1-Sulpiride also induced a selective desensitization of cortical ß-receptors; this effect was not detectable in the striatum which contains a high density of ß receptors, but lacks norepinephrine (NE) innervation. Cortical NE terminals may be endowed with D-2 receptors controlling NE release and blockade of this endogenous DA inhibitory modulation may be involved in the antidepressant effects of 1-sulpiride.

539.4

NEUROLEPTIC COTREATMENTS WHICH ATTENUATE THE DEVELOPMENT OF BEHAVIORAL HYPERSENSITIVITY. L.C. Kao, R. Singh, H.L. Klawans*, P.M. Carvey. Rush Medical College, Chicago, IL 60612.

Rats, chronically treated with neuroleptic agents, develop an increased stereotypical behavioral response to a subsequent challenge with the dopamine (DA) agonist apomorphine (APO). DA receptor proliferation is widely believed to be responsible for this behavioral hypersensitivity (BH). We have studied a variety of cotreatment paradigms to examine the relationship between BH and DA receptor proliferation. Rats were treated for two months with haloperidol (HAL, 0.75 mg/kg) and a variety of cotreatments which included amantadine (AMAN), lithium (Li), scopolamine (SCOP), thioridazine (THIO), or clozapine (CLZ). Four days following the last treatment the animals were challenged with APO and 2 days later they were sacrificed. The striata were examined for alterations in D-2 receptor number using quantitative autoradiography. AMAN, Li, SCOP, and THIO cotreatments significantly attenuated the development of BH to HAL. SCOP cotreatment also attenuated the development of BH induced by fluphenazine. CLZ, however, failed to attenuate HAL-induced BH. AMAN and Li cotreatment prevented the D-2 receptor proliferation normally induced by HAL while SCOP, THIO and CLZ cotreatments did not. Therefore, D-2 receptor proliferation can exist in an animal that does not exhibit BH suggesting that factors in addition to D-2 receptor proliferation participate in the expression of BH. D-2 receptor proliferation is therefore permissive for the expression of BH.

539.5

Modulation of Striatal Dopamine (D2) Binding by Oral Etonitazene and its Antagonism by Chronic Naltrexone. J.B. Fishman* and K.R. Carlson. Dept. of Pharmacology, U. Mass. Med. Ctr., Worcester, MA 01655

Male Sprague-Dawley rats were implanted s.c. with Alzet osmotic minipumps containing either naltrexone (NTX; 70 mg/ml) or saline (SAL). Half of each group was then given free access to the potent opiate etonitazene (ET; 2 ug/ml) or water for 12 days. ET consumption of the SAL-implant rats gradually rose from 30 to 60 ml/day, but this effect was blocked by NTX. Striatal synaptosomes showed increased high affinity binding of [^{3}H]-raclopride to D2 receptors from SAL animals who ingested ET, with no significant change in B_{max}. These changes were abolished by NTX. D1 receptor binding was unchanged. Computer modeling was consistent with the high affinity D2 binding following chronic ET ingestion representing 10-20% of the D2 receptors having a 50- to 100-fold higher affinity. Since antagonists were used for binding and competition, it is unlikely that the modulation of binding is due to changes in receptor/G-protein interaction. (Supported by USPHS DK 39328, DA 06539 and ONR)

539.6

CHANGES IN BASAL GANGLIA DOPAMINE (DA) SYSTEMS AFTER NEONATAL INTRASTRIATAL 6-HYDROXYDOPAMINE (6-OHDA) INJECTIONS. B.S. Neal and J.N. Joyce. Departments of Psychiatry and Pharmacology, University of Pennsylvania School of Medicine, Philadelphia, PA 19104.

It is known that early lesions of DA systems result in different behavioral effects compared to adult lesions. Nonselective damage to DA systems in the early postnatal period results in enhanced responses to D1 agonists, but not to D2 agonists. Thus, there may be differences in DA receptor regulation depending upon the maturity of the system when lesioned. To selectively destroy the DA input to the patch (striosome) compartment, while leaving that to the matrix relatively intact, we administered 6-OHDA bilaterally into the striatum (4 µg per striatum) on day of birth (P0) or postnatal day 1 (P1). The rats were supersensitive to the behavioral effects of D1 agonists and subsensitive to the effects of D1 antagonists as adults. Our aim was to determine if DA receptor changes within the striatum could account for these effects. Quantitative autoradiography on coronal sections of brain tissue from rats given bilateral 6-OHDA lesions revealed a significant and patchy loss (30-50%) of presynaptic DA uptake sites (^{3}H-mazindol), with greater losses in the dorsomedial and dorsolateral striatum. Unlike adult lesions, there were no changes in the density of D2 sites (^{3}H-spiroperidol); however, there was a significant and heterogeneous loss (10-20%) of D1 sites (^{3}H-SCH23390). Decreases (40-50%) in the number of µ-opioid receptor (^{3}H-naloxone) patches were also apparent. A second set of neonatally-lesioned rats was killed on P7 (before the patch/matrix organization is obscured) in order to determine if there was a selective loss of D1 receptors in the patch compartment. Preliminary results suggest that this is the case. Tyrosine hydroxylase immunocytochemistry is also being used to examine the integrity of the patch and matrix-directed DA inputs in these animals. (Supported in part by USPHS Grant MH43852.)

539.7

UP-REGULATION OF DOPAMINE D-1 RECEPTORS IN RHESUS MONKEYS AND RATS FOLLOWING CHRONIC ADMINISTRATION OF SCH 39166, A D-1 SELECTIVE ANTAGONIST. R.A. Duffy*, G. Kaminska*, R.E. Chipkin and R.D. McQuade, Schering-Plough Research, Bloomfield, New Jersey, 07003.

It has been demonstrated that chronic administration of selective neurotransmitter antagonists produces an increase, or up-regulation, in the density of the specific receptor (Bmax). A novel D-1 antagonist, SCH 39166 ((-)-trans-6,7,7a,8,9,13b-hexahydro-3-chloro-2-hydroxy-N-methyl-5-H-benzo-[d]-naphtho{2,1b}azepine) was examined for its ability to produce such a change in Bmax when administered chronically. Adult rhesus monkeys received daily oral injections of either vehicle or SCH 39166 (3, 12 or 48 mg/kg) for three months. The caudate nucleus and putamen were dissected and the D-1 and D-2 receptors were analyzed for changes in their affinities (Kd) and densities. In the putamen, a significant increase in Bmax was seen for all three doses of SCH 39166 (58, 92 and 100% above control, respectively, $p < 0.05$, Duncan's multiple range test), while in the caudate nucleus only the 12 and 48 mg/kg doses produced a significant up-regulation when compared to vehicle controls (50 and 80%, respectively). No significant increase in the density of D-2 receptors was observed in either region.

Similar studies were performed in male rats that had received 50 mg/kg SCH 39166 orally for 7 consecutive days. Analysis of striatal membranes from these groups indicated that SCH 39166 produced a significant increase in the density of D-1 receptors (33% over control animals), without affecting the density of D-2 receptors.

These data demonstrate that in vivo, SCH 39166 is capable of crossing the blood-brain barrier and binding to D-1 receptors in rodents and primates. Further, the selective up-regulation of these receptors suggests that, in vivo, SCH 39166 binds to D-1 receptors without significant interaction at D-2 sites.

539.8

BRAIN *SIGMA* AND DOPAMINE RECEPTORS ARE NOT MODULATED BY CHRONIC D-PENTAZOCINE ADMINISTRATION IN RATS. A.D. Weissman and E.B. De Souza. Neurobiology Laboratory, NIDA Addiction Research Center, Baltimore, MD 21224.

Schizophrenia has been associated with alterations in the regional brain densities of σ and D_2 dopamine receptors. Recent studies have suggested that drugs with high affinities for the σ receptor may produce psychotomimetic effects by modulation of dopamine release. Long-term treatment of animals with σ drugs can regulate the number of σ binding sites. We hypothesized that chronic administration of a drug such as d-pentazocine, with high affinity and specificity for σ receptors, could alter the number of σ binding sites and indirectly affect basal dopamine release. This, in turn, should modulate the number of D_2 receptors. Rats (n = 6/group) were implanted s.c. with mini osmotic pumps to deliver either d-pentazocine (10 mg/kg/day) or saline for 4 weeks. Saturation studies using [^{3}H]YM-09151-2 or [^{3}H]haloperidol in the presence of 50 nM spiperone to label D_2 and σ sites, respectively, revealed no significant changes in the K_D or B_{max} of either site in nine brain regions of drug treated animals. The inability of the specific σ compound d-pentazocine to alter σ receptors differentiates this drug from compounds such as d-3-PPP and DTG that up-regulate σ binding, and from haloperidol, which down-regulates σ sites. Unlike d-pentazocine, the compounds that are able to regulate σ receptors bind to multiple sites. This suggests that chronic interaction with the haloperidol-sensitive site alone may not be sufficient to modulate σ or dopamine binding.

539.9

OPPOSING EFFECT OF CHOLECYSTOKININ OCTAPEPTIDE ON SPECIFIC DOPAMINE D1/D2 TYPE BINDING SITES IN RAT BRAIN. A.M. Kask*, C. Marin, L. Steardo, T.N. Chase, ETB, NINDS, Bethesda, MD 20892.

Blockade of either dopamine (DA) D1 and/or D2 receptors induces catalepsy in rats. Cholecystokinin-octapeptide (CCK8) affects central dopaminergic function, possibly by modulating DA interactions at both D-1 and D-2 receptor sites. The present study was designed to explore the effects of acute intrastriatal injection of CCK8 on D-1 and D-2 receptor mediated catalepsy and the binding capacities of DA receptors at the time of maximal behavioral response. Over a period of 2 minutes, CCK8 (50-800 ng) was injected into each striatum through surgically implanted cannulae. For catalepsy induction, rats received either the D-1 antagonist SCH 23390 (0.5 mg/kg sc), the D-2 antagonist raclopride (RAC, 2.5 mg/kg ip), or saline. Catalepsy was scored for the next two hours. Other rats were sacrificed 20 min after antagonist injection, when catalepsy peaked. Brains were removed and P2 membrane fractions prepared for ex vivo receptor assay. CCK8 significantly diminished SCH induced catalepsy but enhanced RAC induced catalepsy. Given alone, CCK8 did not affect motor behavior, but did increase the number of available receptors of both sub-types . The presence of SCH or RAC alone, reduced the number of respective binding sites available; intrastriatal CCK8 increased the number of available D-1 and decreased the number of available D-2 binding sites. The opposing effect of CCK8 on D1/D2 receptor binding and catalepsy may provide a mechanism for the modulation of the functional interaction of the dopaminergic system by CCK8 either directly or via one of its metabolites.

539.10

EFFECT OF AVP ADMINISTRATION AND AVP DEFICIENCY UPON ^{3}H-AVP BINDING SITES IN CNS AND PERIPHERY. P. Szot and D.M. Dorsa. GRECC, Seattle VAMC, WA 98108.

AVP (40 ug/100gbw, sc) was administered to Long-Evans (LE) pups from day 1-7 of life and sacrificed on day 8 or day 60. No significant changes were observed in ^{3}H-arginine^8-vasopressin (^{3}H-AVP) binding in the kidney, septum or cingulate gyrus of day 8 pups from control. No significant changes were observed in kidney or septum of day 60 animals. However, the chronic AVP treatment did result in a significant increase in the density of ^{3}H-AVP binding sites in the liver when compared to control in day 8 pups (control 44 ± 2; AVP 56 ± 3 fmol/mg protein) and day 60 rats (control 186 ± 9; AVP 239 ± 14 fmol/mg protein). The affinity in liver ^{3}H-AVP binding site remained unchanged for day 8 and 60 animals. This demonstrates a permanent alteration in the density of ^{3}H-AVP binding sites in the liver due to an early postnatal AVP treatment. A comparison of ^{3}H-AVP binding sites in 8 day old LE, heterozygous Brattleboro rat (BB-HET) and homozygous Brattleboro rat (BB-HOM) was performed to assess the effect of complete (BB-HOM) and partial (BB-HET) AVP deficiency on AVP binding sites in the CNS and periphery. The liver again was the only tissue that showed a change in ^{3}H-AVP binding characteristics. The BB-HOM rat (B_{max}=144 ± 6 fmol/mg protein) displayed a significant increase in AVP binding sites from the LE rat (B_{max}=100 ± 7 fmol/mg protein), while the ^{3}H-AVP binding sites in the BB-HET (B_{max}=69.8 ± 9 fmol/mg protein) liver were significantly lower than the LE rat. Thus, hepatic AVP receptors appear most sensitive to the presence or absence of AVP during the early postnatal period.

539.11

KINETICS OF NICOTINE BINDING TO BRAIN TISSUE AFTER CHRONIC NICOTINE INFUSION. R.V. Bhat, S.L. Turner*, M.J. Marks and A.C. Collins. Sch. of Pharmacy, Inst. for Behav. Genetics, Psychology Dept., Univ. of Colorado, Boulder, CO 80302.

The basic model for desensitization of the nicotinic receptors from *Torpedo californica* can be viewed as two conformational states of the receptor having differential affinities for the agonist. Although less studied, it has been suggested that a similar situation occurs with the nicotine binding sites in the brain since receptors in fast (Bf) and slow (Bs) phases of association binding can be detected. Chronic nicotine treatment increases the number of [^{3}H]nicotine binding sites in rodent brain and in decreased sensitivity to nicotine. A conformational shift from a low affinity ground to a higher affinity desensitized form may be responsible for this adaptive effect. Thus, chronic nicotine treatment should cause a shift in the ratio of Bs to Bf binding. C57BL/6 mice were continuously infused with saline or 0.5, 1, 3, 6 mg/kg/hr nicotine for 7 days. The kinetics of [^{3}H]nicotine binding to brain membranes obtained from these mice revealed biphasic association and a monophasic dissociation kinetics. Chronic nicotine treatment did not alter the Bs:Bf ratio. Since the rate of resensitization of the receptors is not known, additional data are necessary to test whether the Bs:Bf ratio is altered *in vivo*. Supported by DA-03194 and DA-00116.

540.1

IN VIVO RELEASE OF ACETYLCHOLINE IN THE BASAL NUCLEAR COMPLEX FROM NEURONS OF THE PONTOMESENCEPHALIC TEGMENTUM (PMT) S. Consolo, R. Bertorelli*, G.L. Forloni and L.L. Butcher+. Istituto "Mario Negri", 20157 Milan, Italy and +University of California, Los Angeles, CA 90024-1563, USA.

In an attempt to determine possible interactions between the basal forebrain (NB) cholinergic complex and the PMT cholinergic neurons, we measured the acetylcholine (ACh) release in vivo from the NB, both alone and in combination with lesion and pharmacologic manipulations. The ACh release was calcium dependent and it was increased by scopolamine (SCOP) (0.5 mg/kg s.c.). The rise in ACh release induced by SCOP (a) persisted in the presence of quisqualate lesions of the NB complex (b) was blocked by tetrodotoxin infusion, and (c) was abolished by ablation of PMT cell bodies. Thus, the calcium dependent ACh release in the NB complex (a) is largely axonal in nature, (b) derives substantially from cholinergic PMT axons, and (c) appears to be controlled by presynaptic muscarinic receptors on PMT axon terminals. Thus, the PMT cholinergic complex might influence cortical ACh release, in part at least, by means of serial-order cholinergic-cholinergic interactions in the basal nuclear complex.

540.2

Effects of an M_1 receptor agonist (AF102B) on the central cholinergic system, evaluated by brain microdialysis. N. Ogane, Y. Takada*, Y. Iga*, G. Kawanishi*, and F. Mizobe. Res. Inst. of Life Sci. Snow Brand Milk Products Co. Ltd., Tochigi 329-05, Japan.

The effects of a novel M_1-receptor agonist, AF102B (FKS-508; cis-2-methylspiro (1,3 - oxathiolane - 5,3') quinuclidine, on the central cholinergic system *in vivo* were evaluated by determination of acetylcholine (ACh) content in the rat brain after microwave irradiation and by measurement of ACh release with microdialysis perfusion in freely moving rats. Intraperitoneal administration of AF102B resulted in a significant decrease of ACh content in the brain, while AF102B produced an increase of *in vivo* ACh release. The present results suggest that ACh content in the brain after treatment with muscarinic agents may be related to the changes of ACh release, in which both M_1 and M_2 muscarinic receptors may be involved.

540.3

MODULATION OF HIPPOCAMPAL ACETYLCHOLINE RELEASE BY NMDA RECEPTORS M.G. Giovannini* and G. Pepeu, Department of Pharmacology, University of Florence, Florence, Italy.

Evidence has been accumulating that the cholinergic and glutamatergic systems of the hippocampus are involved in cognitive processes. While the neuroanatomical organization of the cholinergic and glutamatergic systems in the hippocampus is well known, little is known of the possible interactions between the two neurotransmitter systems. The aim of the present study was to investigate the effect of the modulation of hippocampal NMDA receptors on the release of ACh in freely moving Wistar male rats by means of a transversal microdialysis probe implanted in the proximity of the CA1 region in the dorsal hippocampi. Perfusion of the microdialysis probe with 500 uM NMDA dissolved in Ringer solution brought about a decrease of ACh release compared to the mean of three basal control values (-48 %, $P < 0.05$, Duncan test). A decrease (- 45 %, $P < 0.05$) was also observed following perfusion with the NMDA antagonist 2-amino-7-phosphonoheptanoate (AP7). On the contrary, i.c.v. injection of AP7 (10 ug/rat) was followed by an increase in ACh release (+79 %, $P < 0.05$). These findings indicate a complex modulation of the cholinergic system by NMDA receptors. Supported by C.N.R. grants.

540.4

ACETYLCHOLINE RELEASE IN THE HIPPOCAMPUS, CORTEX AND STRIATUM OF RATS CORRELATES WITH LOCOMOTOR ACTIVITY: AN IN VIVO MICRODIALYSIS STUDY. J. DAY, G. DAMSMA, H.C. FIBIGER. Div. of Neurological Sciences, Dept. of Psychiatry, Univ. of British Columbia, Vancouver, B.C. V6T 1W5

Simultaneous measurements of locomotor activity and dialysate concentrations of acetylcholine (ACh) and choline (Ch) in the dorsal hippocampus, frontal cortex, and dorsal striatum of rats were made under three conditions: 1) drug-free, after the administration of vehicle (H_2O); 2) after administration of the muscarinic receptor antagonist, scopolamine (0.4 mg/kg); and 3) during the change from light to dark in the rats' daily cycle. Vehicle injections increased ACh concentrations in the cortex and hippocampus, but not in the striatum. Scopolamine stimulated locomotion and increased extracellular concentrations of ACh to 404% (striatum), 1241% (hippocampus) and 1435% (cortex) of basal values. Ch concentrations in the hippocampus and cortex were lowered slightly by scopolamine; Ch in the striatum was transiently increased by this drug. Within 20 minutes after dark, ACh increases of 58%, 77% and 169% were measured in the striatum, cortex and hippocampus, respectively. Locomotor activity also increased during this time. Dark exposure did not change Ch in any area. Significant positive correlations between ACh release and locomotor activity were found for each brain region under all three conditions. These results indicate that cholinergic transmission can undergo regionally selective changes in response to environmental and pharmacological stimuli.

540.5

AMPHETAMINE-INDUCED INCREASES IN STRIATAL ACETYLCHOLINE RELEASE AS MEASURED BY MICRODIALYSIS ARE NOT DEPENDENT ON NIGROSTRIATAL DOPAMINE. R.J. MANDEL, O.G. NILSSON*, E. ROSENGREN*, and A. BJÖRKLUND. University of Lund, Dept. of Medical Cell Research, Biskopsgatan 5, S-223 62 Lund, Sweden.

One of the most consistent findings in the study of striatal dopamine (DA) receptor pharmacology is the inhibitory control by D-2 receptor stimulation of striatal acetylcholine (ACh) release *in vitro*. The present study was undertaken to characterize the *in vivo* dopaminergic control of ACh release in the rat striatum. In preliminary experiments, we observed a consistent 100-200% increase in striatal ACh release in response to 5 mg/kg systemic amphetamine (AMPH, ip) but no effect when AMPH was administered locally in the probe (10 µM AMPH, with 5 µM neostigmine, 15 min samples). Since the systemic data are in direct contrast to many reports using the *in vitro* slice technique, an experiment was undertaken to determine whether the effect was dependent on striatal DA. Eleven rats were administered unilateral 6-hydroxydopamine lesions with 5 receiving desmethylimipramine to protect noradrenergic striatal projections. All rats were implanted with bilateral loop-type microdialysis probes in the striatum (AP +0.7 mm, LAT ±2.6, DV -6.0, from bregma and dura). There was no effect of lesion on either baseline or AMPH-induced striatal ACh release with systemic AMPH causing a 100-200% increase compared to baseline levels. A preliminary experiment (n = 3) utilizing an extremely ventrolateral striatal placement of the dialysis probe in intact rats also indicates a large increase in striatal ACh release in response to systemic AMPH. These data suggest that local striatal release of DA is not responsible for the observed effect of AMPH on ACh release and there is no differential dopaminergic control of striatal ACh in these striatal subregions. Thus, some multisynaptic pathway, perhaps that can be influenced by the opposite nigrostriatal DA projection, must be responsible for the effects reported here.

540.6

NEUROTENSIN REGULATES ACETYLCHOLINE RELEASE FROM STRIATAL INTERNEURONS: EFFECT OF 6-OHDA LESIONS OF THE SUBSTANTIA NIGRA. R. Quirion, A. Beaudet, D.M. Araujo and P.A. Lapchak. Douglas Hosp. Res. Ctr. and Neuroanat. Lab., Mtl. Neurol. Inst., McGill Univ., Montreal, Canada.

Neurotensin (NT) immunoreactivity and receptors are present in the striatum, a region enriched with dopaminergic and cholinergic innervation. The present study determined whether NT alters the activity of striatal cholinergic interneurons and if nigral DA neurons modulate this effect. NT (10 µM) increased K^+-evoked (50%), but not basal, ACh release from striatal slices. The effect was concentration-dependent and tetrodotoxin-insensitive. In the presence of the dopamine antagonist sulpiride (50 µM), the effect of NT (10 µM) was further enhanced by an additional 40%. In slices from 6-OHDA lesioned rats, in which striatal ChAT activity was not changed, but basal and evoked ACh release were increased (33% and 40%, respectively), the effect of NT on evoked ACh release was similar to control (48% increase). In addition, in lesioned animals, sulpiride (50 µM) did not alter evoked ACh release, nor did it augment the effect of NT (10 µM) alone. These results suggest that in the striatum, NT regulates ACh release by a direct action on cholinergic elements. (Supported by MRC, Canada and FRSQ, Quebec).

540.7

PHARMACOLOGICAL STUDIES OF ACETYLCHOLINE RELEASE BY MICRODIALYSIS IN THE FREELY MOVING RAT. L.K. Gorman, D.S. Olton, S. Plano* and G.L. Wenk. Neuromnemonics Lab., Dept. of Psychology, The Johns Hopkins Univ., Baltimore, MD 21218.

Sodium dependent high affinity choline uptake levels are increased following i.p. injections of scopolamine or pentylenetetrazol, and decreased by pentobarbital. This study examined the effects of these pharmacologic manipulations on two other measures of cholinergic activity, ACH release and the number of high affinity choline uptake (HACU) sites. Changes in release of ACH were determined by in vivo microdialysis. Six 5-minute microdialysate samples, from the hippocampus and overlying cortex, were collected to obtain baseline levels prior to drug injection and an additional twelve successive dialysate samples were collected after drug injection. Levels of choline and ACH were assayed using HPLC with electrochemical detection. One hour prior to the determination of HACU binding by hemicholinium-3, each rat was given an injection of one of the three drugs. A correlation between the kinetics of ACH release and HACU binding following drug injections that alter the activity of the cholinergic system can be determined using this technique. Supported by NSF BNS 88-07010 and NIH NS 20471.

540.8

EFFECT OF THE PHYSOSTIGMINE DERIVATIVE HEPTASTIGMINE ON ACETYLCHOLINE RELEASE ASSESSED BY MICRODIALYSIS IN RAT BRAIN. E. Messamore, N. Ogane, E. Giacobini and E. Williams*. Dept. of Pharmacology, Southern Illinois Univ. Sch. Med. Springfield, IL 62974, USA

Heptastigmine [heptyl-physostigmine(MF-201), Mediolanum Farm., Milan, Italy] is a new derivative of the carbamate acetylcholinesterase (AChE) inhibitor physostigmine (Phy) with lower toxicity and longer inhibitory action on brain AChE than the parent compound. Following single dose administration of 5 mg/kg i.m. an 80% inhibition of brain AChE is reached at 60 min; at 360 min inhibition is still 67%. Increases in acetylcholine (ACh) levels measured in brain homogenates are seen in all brain regions with a maximum increase of 60% occurring in striatum 2h after injection. Local AChE inhibition by Phy as well as MF-201 administered via microdialysis probe elevates ACh recovered from striatum in a dose-dependent manner. In contrast to the ACh elevating effect of AChE inhibition observed in the aforementioned studies, systemic administration of MF-201 (5 mg/kg) decreases ACh recovered by microdialysis from the striatum of freely moving rats; ACh falls to 84% of its baseline value 2 hours after i.p. injection. Microdialysis permits recovery of ACh from the extracellular space, while assay of brain homogenate reflects extracellular as well as intracellular compartments. MF-201 decreases ACh measured by microdialysis yet increases ACh measured in brain homogenate. Therefore ACh elevation in brain homogenate following a high level of AChE inhibition reflects a predominantly intracellular drug effect.

540.9

EFFECT OF TRH AND A TRH ANALOGUE YM-14673 ON EXTRACELLULAR ACH LEVELS OF RAT IN FREELY MOVING CONDITIONS

M.Okada* and T.Yamaguchi Central Res. Labs., Yamanouchi Pharmacetical Co. LTD., 21 Miyuki-gaoka, Tukuba, Ibaraki, 305, Japan.

TRH and YM14673 has been reported to possess analeptic actions such as shortening effects on pentobarbitone-induced sleeping time in rodents. These effects were inhibited by anti-muscarinic agents, suggesting the involvement of stimulation of cholinergic system. Therefore, using in vivo brain dialysis combined with HPLC-ECD system, we have measured flux of ACh induced by TRH and YM-14673 in the frontal cortex(FC) and the caudate nucleus(CN) of freely moving rat.

In the FC, TRH(10 mg/kg i.p.) increased the extracellular ACh by 250% reaching a peak at 40 min post drug. YM-14673 produced a dose-dependent increase in ACh levels. YM-14673 at 0.1 mg/kg i.p. enhanced ACh release by 220% reaching a maximum at 60 min and lasting for 40 min. While, in the CN, a higher dose of YM-14673(3 mg/kg i.p.) increased the extracellular ACh levels by only 25%. These results suggested that a part of the CNS-stimulating action of TRH and YM-14673 might be ascribed to the increase of ACh release in the cortex.

540.10

DIISOPROPYLFLUOROPHOSPHATE IS NOT A RELIABLE AGENT FOR THE STUDY OF CHOLINERGIC FUNCTION. A.W. Kirby & A.T. Townsend*. U.S. Army Aeromedical Research Laboratory, Ft. Rucker, AL 36362.

Diisopropylfluorophosphate (DFP) is an irreversible inhibitor of cholinesterase (ChE), and is used widely to investigate cholinergic function. Recent results have suggested that DFP might have significant actions totally independent of cholinergic circuitry.

All experiments were done on fully anesthetized adult cats. Release of labeled DA, GABA, and glycine from isolated retina was increased by DFP exposure, but not by physostigmine or excess ACh as would be expected if DFP were acting solely on cholinergic mechanisms. The increase persisted with exposure to atropine or mecamylamine, and without synaptic transmission, suggesting a direct effect of DFP on retinal neurons. Direct application of DFP to the cortical surface caused changes in the visual evoked response and cortical neurochemistry, similar, but of less magnitude than those resulting from i.v. DFP. A direct effect of DFP on cortical neurons, independent of or in addition to cholinergic effects, could explain these changes. Because of apparent noncholinergic actions of DFP, other anticholinesterase agents should be used to validate DFP results.

540.11

EFFECTS OF TRIHEXYPHENIDYL (TH) AND BIPERIDEN (BP) ON BRAIN NEUROTRANSMITTER LEVELS OF RAT INTOXICATED WITH SOMAN.

T.-M. Shih, B. Capacio* L. Cook*, T. Koviak*, T. Sewell* and N. Adams*. U.S. Army Med. Res. Inst. Chem. Defense, Aberdeen Proving Ground, MD 21010-5425.

It has been reported that antimuscarinics possessing antiparkinson activity are effective in preventing convulsions induced by the cholinesterase (ChE) inhibitor soman. The purpose of this study was to see if the drugs, given to rats at an anticonvulsant dose of 0.125 mg/kg, im, would cause changes in cholinergic or catecholaminergic parameters in the striatum of rats poisoned with soman (100 ug/kg, sc). The time courses (up to 2 hr) for ChE activity and levels of acetylcholine (ACh) and catecholamine were measured after soman, TH, BP, soman + TH, or soman + BP treatment. Soman inhibited ChE activity (60%; 15 min) and increased ACh levels (35%; 15 min). It did not affect norepinephrine or dopamine (DA), but elevated levels of the DA metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), thus indicating increased DA turnover. BP and TH alone did not affect any of the neurochemical parameters studied. TH and BP each significantly reversed the effects of soman on DOPAC and HVA levels, but neither affected cholinergic parameters. Our data suggest that, in addition to actions on muscarinic receptors, the anticonvulsant effects of TH and BP in soman poisoning may be partially related to their actions on the striatal dopaminergic system.

540.12

EFFECT OF THE CHOLINESTERASE INHIBITOR HUPERZINE-A ON ETHYLCHOLINE AZIRIDINIUM (AF64A) CHOLINOTOXICITY IN THE RAT.

S. Laganiere, J.C. Corey*, X.C. Tang*, E. Wulfert* and I. Hanin. Dept. Pharmacology, Loyola University Chicago Stritch School of Medicine, Maywood, Il 60153; and UCB s.a.*, Pharmaceutical Sector, Brussels, Belgium.

Huperzine-A (Hup-A) is a natural and long lasting acetylcholinesterase (AChE) inhibitor isolated from a *Lycopodium* found in China. Hup-A was reported to elevate levels of acetylcholine in rat brain by up to 40% for several hours and has been proposed as a palliative treatment in Alzheimer's disease (Tang et al., J. Neurosci. Res. 24:276, 1989). Previous studies on chronic treatment with Hup-A (0.5 mg/kg, twice daily for 4.5 days) showed a 19 to 30% reduction of AChE in various areas of the brain. We also observed a 25% transient inhibition of high affinity choline transport (HAChT) in hippocampal synaptosomes 45 min. after injection. However, the inhibition was completely recovered at 90 min. This effect was secondary to (AChE) inhibition, since Hup-A had no effect on HAChT in hippocampal synaptosomes *in vitro*.

Hemicholinium-3, a potent inhibitor of HAChT, has been shown to protect efficiently against AF64A hippocampal cholinotoxicity *in vivo*. We thus explored whether the reduction of HAChT in the Hup-A-treated rat would also protect against AF64A cholinotoxicity. Male Sprague-Dawley rats were pre-treated for 4.5 days with Hup-A (i.p., 0.1 or 0.5 mg/kg, twice daily). Bilateral administration of AF64A (2 nmol/ventricle icv; 3 ul/3 min) was performed within 45 min after the 9th pre-injection. Hup-A was continued (C) or withheld (W) in groups of AF64A-treated rats, for 6 days, until sacrifice. In Hup-A treated rats, results clearly indicated that hippocampal HAChT activity was 30% ($p<0.03$) lower than in AF64A-injected controls. AChE activity was also significantly lowered by 22%, but only in the W groups. Levels of choline acetyltransferase were not further decreased by Hup-A. The usual absence of striatal cholinotoxicity following AF64A was not modified. In conclusion, Hup-A-induced reduction in hippocampal HAChT was not sufficient to protect against AF64A cholinotoxicity *in vivo*. Supported by NIA Grant #AG07591 and by UCB s.a., Brussels.

540.13

DOSE-TIME EFFECT OF ETHYLCHOLINE AZIRIDINIUM (AF64A) ON CYCLIC AMP (cAMP) LEVELS IN RAT BRAIN: CORRELATION WITH THE REVERSIBILITY OF CHOLINERGIC DISRUPTION AT LOW DOSES OF AF64A. A. El-Tamer*[•§], E. Wulfert*[§], and I. Hanin[•]. Loyola University Chicago Stritch School of Medicine, Maywood, Illinois 60153[•], and UCB s.a., Pharmaceutical R&D, Brussels, Belgium[§].

AF64A-induced reduction in cholinergic function may, through a possible feedback loop, involve noradrenergic mechanisms (Hortnagl et al., J. Neurosci. Methods 27: 103, 1989). We wondered whether the second messenger cAMP might reflect these AF64A-induced catecholaminergic changes. Animals were treated with various doses of AF64A (0.25, 0.5, 1.0 and 2.0 nmol) icv, bilaterally, and killed by decapitation at 2, 4, 7, 14, 28 and 42 days after such treatment. Hippocampus, striatum and frontal cortex were rapidly dissected out and tissues were subsequently extracted in 6% TCA. cAMP was measured by radioimmunoassay. Choline acetyltransferase (ChAT) and acetylcholinesterase (AChE) activities were also measured in a portion of the same tissues, employing conventional radioenzymatic techniques. Changes in cAMP, ChAT and AChE were observed only in the hippocampus, thus indicating region-specific effects of AF64A. By 2 days following AF64A treatment, at all doses tested, there was a dose dependent (23% -36%) decrease in hippocampal cAMP levels, respectively. By 7 days, all values returned to normal. Hippocampal ChAT and AChE reductions following doses of 0.25 and 0.5 nmol/ventricle reached a nadir (25% - 60%) by 4 and 7 days, respectively, but returned completely to normal values within 14 days following AF64A treatment. ChAT and AChE levels following treatment with 1.0 and 2.0 nmol AF64A/ventricle were reduced significantly by 4 days, and did not recover even 42 days post AF64A. These combined data imply a modulatory role for cAMP dependent mechanisms in the disruption of central cholinergic function induced by AF64A treatment.

540.14

AF64A DOSE-DEPENDENTLY BUT NON-UNIFORMLY DECREASES THE NUMBER OF CHOLINE ACETYLTRANSFERASE (ChAT) IMMUNOREACTIVE (IR) CELL BODIES IN THE SEPTAL COMPLEX. S.A. Lorens, G. Kindel*, X.W. Dong*, J.M. Lee and I. Hanin. Dept. Pharmacology, Loyola University Chicago Medical Center, 2160 S. First Ave., Maywood, IL 60153.

The cholinotoxin, AF64A (0.5,1.0 or 1.5 nmole/ventricle), or vehicle (3.0 ul) was injected bilaterally into male F344 rats (n=23). ChAT-IR perikarya in the four subgroups of the septal complex (Amaral, D.G. & Kurz, J., J. Comp. Neur., 240:37-59, 1986) were visualized (40 um sections at 0.2 mm intervals; PAP technique) 28 days post-injection and counted using a microprojector (x25). The 0.5 nmole dose of AF64A significantly reduced (31%) the number of ChAT-IR cell bodies in the intermediate subgroup (rostral extension of nucleus basalis). Higher doses did not produce additional reductions. The largest AF64A dose resulted in significant reductions in ChAT-IR cell bodies in the dorsal (51%) and midline (35%) subgroups (medial septum), but did not affect the number of ventral subgroup (horizontal limb of the diagonal band) ChAT-IR neurons. Electrolytic lesions were placed in 6 rats in order to produce non-selective damage to the corpus callosum, cingulum and overlying cingulate gyrus, similar to that seen following higher doses of AF64A. These lesions failed to significantly affect the number of ChAT-IR cell bodies in any of the septal subdivisions. These results suggest that the distinct subgroups of septal ChAT-IR neurons are differentially sensitive to the toxic effects of i.c.v. administered AF64A: intermediate >> dorsal > midline >> ventral subgroup. AF64A may diffuse into the cingulate gyrus and hippocampal formation where it is taken up by cholinergic terminals and produces retrograde degeneration of septal cell bodies. Alternatively, AF64A may spread directly to the septal complex where it is transported into cholinergic cell bodies and induces cytotoxicity, the cholinergic perikarya in the intermediate cell group being the most sensitive. Supported by Grant #MH42572.

540.15

DECREASED CHOLINE ACETYLTRANSFERASE ACTIVITY AND ^{3}H-ACETYLCHOLINE RELEASE IN THE RAT MEDIAL SEPTAL/ DIAGONAL BAND COMPLEX AFTER FIMBRIA-FORNIX TRANSECTION. R.H. METCALF and R.J. BOEGMAN. Department of Pharmacology and Toxicology, Queen's University, Kingston, Ontario, Canada K7L 3N6.

We have previously shown that ACh release in the medial septal/diagonal band complex (MSDB) may be stimulated by 35 mM potassium (K^+). However, the origin of this release is unknown. Electrophysiological and morphological data suggest that the release may be from local collaterals originating from cholinergic neurons projecting to the hippocampus. Transection of the fimbria-fornix pathway produces retrograde losses of MSDB cholinergic markers. Studies were undertaken to determine if FF transection would alter ChAT activity and/or ^{3}H-Ach release as well as AchE histochemistry in the MSDB. Rats received complete FF transections and then were allowed to recover for 1, 3, and 5 weeks. AchE histochemistry 1 week following transection revealed an abundance of swollen AchE-positive fibers in the MSDB which by 3 weeks had disappeared, along with a large majority of AChE-positive cell bodies. This loss was still evident at 5 weeks. ChAT activity measured in the hippocampi and MSDB of non-operated controls was 65.4 ± 7.3 and 137 ± 10.7 nmoles/mg protein/hr respectively. When compared to normal animals, ChAT activity in the hippocampus decreased by 80% 1 week following transection. In the MSDB, ChAT activity decreased by 20% 1 week following transection and was decreased by 32% at 3 weeks. This decrease was still present at 5 weeks. In normal animals, 35 mM K^+ produced an 11.2 ± 1.9 fold increase in MSDB ^{3}H-ACh release. One week following transection, ^{3}H-ACh release was the same as that observed in control experiments. At 3 weeks, a 50% decrease in release was observed in the MSDB. ^{3}H-ACh release was still decreased at 5 weeks. These results suggest that a portion of cholinergic activity seen in the MSDB may be due to local collaterals of cholinergic neurons projecting to the hippocampus. Supported by the MRCC & OMHF.

540.16

RECOVERY OF ACH RELEASE AND ACH CONTENT FROM AH5183 INHIBITION IN HIPPOCAMPAL SLICES. R. Cabeza and B. Collier. Department of Pharmacology, McGill University, Montreal, Quebec, Canada, H3G 1Y6.

2-(4-phenylpiperidino)cyclohexanol (AH5183) inhibits vesicular ACh uptake and ACh release. Though all cholinergic vesicles are sensitive to AH5183, their sensitivities differ. We investigated the recovery of the subcellular ACh contents following treatment with AH5183 to see if different fractions recover at different rates. Hippocampal slices were exposed to the drug, stimulated, and allowed to recover for 10 to 50 min by removing AH5183 and exposing the slices to ^{3}H-Ch. At each time point slices were processed for the determination of subcellular ACh and ^{3}H-ACh contents. Cytoplasmic ACh (S_3 fraction) declined after 40 min while that of the occluded pools (P_3 fraction) peaked at 30 min. By 50 min both fractions were not different from controls. The specific activity (S.A.) of the S_3 fraction increased with time where as that of the P_3 plateaued at 30 min. Since the P_3 fraction is an amalgamation of several fractions it was further separated to see if differences in the recovery of the occluded pools existed. Though the D fraction is most sensitive to the drug's inhibition, the ACh of all fractions increased at the same rate and peaked at 30 min. Interestingly, the D fraction's increase in ACh content did not include ^{3}H-ACh for its ACh S.A. remained at or near 0 for all the time points. (supported by M.R.C. and TS-MH-18882-01).

540.17

EFFECTS OF CHOLINE AND FETAL SEPTAL GRAFTS ON SOME CHOLINERGIC PARAMETERS IN FIMBRIA-FORNIX LESIONED RATS. İ. H. Ulus, E. Korfalı,* S. Uysal,* V. Savcı*, N. Demiröz* and B. K. Kıran*. Departments of Pharmacology and Neurosergury, Uludağ Univ. Med. Sch., Bursa, Turkey.

A 2x3 mm cavity was made by suction unilaterally on the surface of right hippocampus, and fimbria-fornix was lesioned in 31 rats under thiopental (45 mg/kg) anesthesia. Two weeks later, the cavity was re-opened and fetal septal grafts were inserted in 15 rats ("septal-grafted"). The remaining 16 rats served as the "sham-grafted" group. Six rats in each group were given choline in their drinking water (15 mg/ml) for period of 8 weeks by starting 5th week of the grafting. 11th week of grafting motor activities of rats were measured, and 12th week of the grafting rats were killed and their brains removed. Choline acetyltransferase (CAT) activity and ^{3}H-QNB binding were assayed in the right hippocampus.

CAT activity, B_{max} for ^{3}H-QNB binding and motor activity were 36%, 71% and 20%, or 74%, 98% and 113% of control in sham- or septal-grafted rats, respectively. Supplementation of choline altered none of these parameters in septal-grafted rats, but restored, greatly, toward normal decreased motor activity in sham-grafted rats.

These results show that long term choline supplementation has no harmfull effect on the repairing action of cholinergic fetal septal grafts, and restores some of behavioural consequence of cholinergic neurones lost.

540.18

IMMUNOHISTOCHEMICAL EVIDENCE THAT A VESAMICOL ANALOGUE REDUCES THE EXPRESSION OF CHOLINE ACETYLTRANSFERASE (ChAT) IN THE RAT BRAIN WITHOUT AFFECTING OTHER NEUROTRANSMITTER-SPECIFIC MARKERS. L. L. Butcher, S. M. Parsons# and P. L. Di Patre. Dept. of Psychology, UCLA, Los Angeles, CA 90024-1563; #Dept. of Chemistry, UC Santa Barbara, CA 93106.

The compound *trans*-2-(4-Phenylpiperidino)cyclohexanol (now called Vesamicol) is known to inhibit the storage of acetylcholine in nerve terminal synaptic vesicles. A number of analogues have been synthesized recently and their potencies evaluated on purified synaptic vesicles from *Torpedo* electric organ (Rogers et al., *J. Med. Chem.*, **32**: 1217, 1989). We tested the effects of intracerebroventricular (i.c.v.) administration of one of these drugs, here called OD72. Adult Sprague-Dawley rats were treated i.c.v. with OD72, 10 nmol or vehicle, and transcardially perfused 7 days after surgery. In OD72-treated rats we found a disappearance of neurons expressing ChAT in the cholinergic nuclei of the basal forebrain on the injected side, while ChAT-positive neurons in the brainstem were not affected. Other cholinergic markers (nerve growth factor receptor, acetylcholinesterase) were not changed. In addition, the number of neurons expressing GABA-like immunoreactivity in medial septum, as well as cells in the *locus coeruleus* immunoreactive for dopamine-*B*-hydroxylase (DBH) was unaltered as a consequence of OD72-administration. No alterations of Nissl stained cells were observed. These data suggest that OD72 might be a drug capable of reducing ChAT expression selectively in the basal forebrain, without affecting noncholinergic markers like DBH or GABA. [Support: NS 10928]

541.1

COLOCALIZATION OF 11β-HYDROXYSTEROID DEHYDROGENASE AND MINERALOCORTICOID RECEPTOR IN RAT BRAIN. R.R. Sakai, V. Lakshmi*, C. Monder*, J.W. Funder*, Z. Krozowski* and B.S. McEwen
The Rockefeller Univ., Lab. of Neuroendocrinology and The Population Council, New York, NY, 10021; and Medical Research Centre, Prince Henry's Hospital, Melbourne 3004, Australia.

The activity of 11β-hydroxysteroid dehydrogenase (11-DH), which converts corticosterone and cortisol to receptor inactive 11-dehydro metabolites, may provide a mechanism which enables aldosterone to gain access to Type I (mineralocorticoid) receptors, despite the concurrent availability of 100-1000 fold higher competitive concentrations of corticosterone. To further test this possibility, we examined the immunocytochemical localization of Typed I receptors and 11-DH in rat brain, with antibodies directed against renal mineralocorticoid receptors and purified hepatic 11-DH. Type I positive cells were localized throughout the brain, complementing their known distribution by receptor binding assay. The hippocampus was the area of highest Type I receptor density, followed by the cortex, paraventricular nucleus of the hypothalamus and amygdala. 11-DH colocalized with Type I immunoreactivity in all brain areas listed above, and was best demonstrated in the hippocampus. These data support the idea that 11-DH is important in mediating the expression of aldosterone specific effects at the Type 1 receptor and further suggest that the action of 11-DH in relation to the mineralocorticoid in the brain may be via an autocrine rather than a paracrine mechanism as is believed to occur in the kidney. Supported by NS 08537(RRS), MH 43787 (BSM), DK 37094(CM) and the NHMRC of Australia (ZK, JWF).

541.2

NEUROPEPTIDE Y AND DYNORPHIN IN THE BRAIN AND PITUITARY GLAND OF A TELEOST FISH. L. M. Cepriano*, M. P. Schreibman and S. Holtzman*. Department of Biology, Brooklyn College, CUNY, New York 11210.

The distribution of neuropeptide Y (NPY) and dynorphin (DYN) in the brain of immature and mature male platyfish (Xiphophorus maculatus), a freshwater teleost, was studied using immunocytochemistry on Bouin's-fixed, paraffin-embedded material. Immunoreactive (ir)-NPY was found in perikarya and nerve tracts of the nucleus olfactoretinalis, telencephalon, ventral tegmentum and in the neurohypophysis and specific cells of the adenohypophysis. Ir-DYN was found in nerve tracts in the olfactory bulb and in association with cells of the pars intermedia and caudal pars distalis of the pituitary gland. The association of NPY and DYN with brain structures and pituitary gland regions previously found to be involved with sexual maturation, growth and differentiation, suggests that these neuropeptides play a role in these events. [Supported by NASA (NAGW-1704), AID, and PSC-CUNY.]

541.3

THE ROLE OF ESTROGEN IN THE SEXUALLY DIMORPHIC DISTRIBUTION OF CHOLECYSTOKININ (CCK) RECEPTORS IN THE RAT BRAIN: A QUANTITATIVE IN VITRO AUTORADIOGRAPHY STUDY. K.D. Richardson Morton, J.S. Ackman* and N.J. MacLusky. Dept. of Reproductive Science, University of Toronto, Toronto, Ontario M5G 1L7.

Quantitative in vitro autoradiography studies have shown sex differences in CCK receptor levels in rat brain. Male and female rats were gonadectomized and implanted (s.c.) after 1 week with either vehicle or 10% estradiol Silastic capsules 3 days prior to sacrifice. Tissue sections (10 um) were preincubated at 25°C for 15 min. with 50 mM Tris-$MgCl_2$ buffer with 0.2% bovine serum albumin, 1mM dithiothreitol,and 0.2% bacitracin (pH 7.7) followed by a 1 hr incubation with 0.1 nmol ^{125}I-CCK-8 in the preincubation buffer. Females showed increased levels of CCK receptors in the hypothalamic ventromedial nucleus (VMN) and the cingulate cortex compared to the males. Estrogen treatment produced significant decreases in CCK receptors in both the VMN and cingulate cortex of female rats, but had no effect in the male rats. These findings are in agreement with Akesson et al. (Neuroendo. 45: 257-262, 1987) who reported that a bolus injection of estradiol 24 hrs prior to sacrifice significantly reduced CCK binding in the VMN in ovariectomized rats. These results suggest a sexually dimorphic distribution of CCK receptors in the rat brain, and a role for estrogen in modulating these receptors in the female rat. Supported by MRC Canada.

541.4

IMMUNOHISTOCHEMICAL LOCALIZATION OF CALRETININ IN RAT FOREBRAIN. L. Winsky and D.M. Jacobowitz, Lab. of Clinical Sciences, NIMH, Bethesda, MD 20892

A specific and sensitive calretinin antibody (Winsky et al., 1989, PNAS 86: 10139) was used to reveal cell bodies, fibers and pathways within rat forebrain. Sections were incubated in calretinin antibody followed by preparation for fluorescence or peroxidase staining. Results revealed calretinin-specific stain of neurons and fibers in several regions including: olfactory bulb (granule cells, periglomerular cells), hypothalamus, amygdala, cerebral cortex and hippocampus (non-principal cells). Several neuronal circuits were also revealed. Fibers of intensely fluorescent lateral mamillary nucleus cells could be traced along the mamillothalamic tract to terminal areas in the anterior medial nucleus of the thalamus. Labeled cells and fibers of the medial habenula projected to the interpeduncular nucleus via the fasciculus retroflexus. The nigrostriatal and mesolimbic systems were also revealed by calretinin antisera. Other regions with particularly dense calretinin stain included the septofimbrial n., medial amygdaloid n. and periventricular, reticular, anterior dorsal and reunions nuclei of the thalamus. These results extend previous findings indicating a high density of calretinin in brainstem sensory regions and reveal a unique localization of this calcium binding protein in forebrain regions of clinical significance.

541.5

THE ONTOGENY OF TRANSFERRIN RECEPTORS IN THE DEVELOPING CHICK RETINA, Arnold G. Hyndman and Sa Sun Cho. Department of Biological Sciences, Rutgers University, Piscataway, NJ 08855.

Transferrin is a growth factor likely to play a significant role in CNS development. In this study, the appearance of the transferrin receptor (TfR) in the developing chick retina is examined immunohistochemically. TfR immunoreactivity is first detectable in the ganglion cells of E (embryonic day) 4 retina. At E10, TfR immunoreactivity appears in the inner nuclear layer and in photoreceptor cells. In contrast to transferrin, which is characteristically located in the plexiform and nerve fiber regions, and in the outer segments of the photoreceptors, TfR is located primarily on soma. TfR immunoreactivity in ganglion cells and the inner nuclear layer decreases during the later stages of embryonic development (E15 to hatching), but remains strong in the photoreceptors. In addition to neurons, Müller cells and glia of the optic nerve fiber layer are TfR positive. The TfR positive glia of the optic nerve fiber layer are distinctive in their morphology, location and developmental pattern. At E6, they appear along the central portion of the optic streak. They have an ovoid soma and either unipolar or bipolar processes. From E6 to E15, these cells increase rapidly in number and can be gradually found in the peripheral regions of the retina. At E15, star shaped TfR positive glia can be observed. These glial cells, in time, become the predominate population of TfR positive glia.

541.6

THE DISTRIBUTION OF TAURINE-LIKE IMMUNOREACTIVITY IN THE AVIAN CENTRAL NERVOUS SYSTEM. D.S. Henshel and J.D. Steeves. Department of Zoology, University of British Columbia, Vancouver, B.C. Canada V6T 3A4.

Taurine, or a taurine-containing dipeptide (γ-glutamyl- or glycyl-taurine) has been implicated as a neuromodulator or inhibitory neurotransmitter in the CNS. We have used a monclonal taurine antibody ("Tau2", kindly donated by K. Magnusson, J. Madl and A. Beitz) to examine the distribution of taurine-like immunoreactivity (TLI) in the brains and spinal cord of paraformaldehyde-perfused chicken (Gallus gallus) and heron (Ardea herodias) hatchlings. Many small and medium sized cells exhibit TLI throughout the brain. In the forebrain, especially in the striatum, hippocampus and parahippocampus, most of the immunoreactive cells appear to be neuronal. In the midbrain, brainstem and spinal cord the most intense TLI is seen in glial cells. In the cerebellum, a variety of cell types are immunoreactive, including glia, basket cells, and some purkinje cells. In general, the few very large neurons which are immunoreactive (i.e. cerebellar purkinje cells and neurons in a few cranial nuclei) have only light positive staining. Moderate to intense TLI is also seen in the pineal. Throughout the brain (white and grey matter) many fibers exhibit light, medium or dark TLI. In the white matter (brain and spinal cord) many small intensely immunoreactive glial cell bodies are seen. At least some of these immunoreactive glia appear to be oligodendroglia. Thus, TLI is found in both neurons and glia and is widely distributed throughout the avian brain.

541.7

Rapid feasability studies of new PET ligands: High resolution PET in small animals. M. Ingvar, L. Eriksson*, S. Stone-Elander*, G. Rogers*, L. Widén*, PET-Section, Karolinska Institute and Hospital, S-104 01 Stockholm, Sweden and Department of Chemistry, University of California Santa Barbara, CA.

The development of sufficient amounts of a radiotracer for use in PET is often a time-consuming process of optimizing radiolabelling yields and handling procedures. Sometimes the radiotracer is not the original drug, but rather a derivative with unknown in vivo biopharmacological properties. We have therefore developed a fast and relatively inexpensive method of testing new ligands in vivo. Small animal studies require relatively low amounts of radioactivity, can be performed without sterility and toxicology tests and may also serve as a preliminary basis for radiation safety calculations since whole body scans can be performed.

We have tested the procedure with an 18-F analog of Vesamicol, a drug that specifically binds in vitro to synaptic vesicles of cholinergic neurons. For the procedure the rats were anesthetised, ventilated and provided with arterial and venous catheters. The animals were placed in the PET scanner during the whole procedure and sequential scans were performed. After a bolus i.v. injection of 50-100 µCi of the ligand repeated arterial samples were taken. The plasma radioactivty was determined to obtain the brain input function. Following image reconstruction of the scans standard time/radioactivity plots were constructed. The camera (Scanditronix PC2048-15B) has a resolution of 4.5 mm FWHM and a rat brain is approximately 16-25 pixels in the 128*128 image. The major advantage of this procedure is that very few experiments are necessary to reliably determine the kinetic properties of the blood brain barrier transport and the magnitude of whole brain binding to a receptor system. In vivo displacement of a ligand from a receptor can be made with potentially toxic amounts of the drug. This is especially important since many receptors are characterized by a state-dependent binding that impairs a direct comparison of in vitro experiments with the in vivo situation.

541.8

PRESENCE OF IMIDAZOLEACETIC ACID RIBOSIDE, A METABOLITE OF IMIDAZOLEACETIC ACID, IN HUMAN CEREBROSPINAL FLUID. G.D. Prell, A.M.Morrishow*, E.Douyon* and S. Jotkowitz*. Department of Pharmacology, Mount Sinai School of Medicine, CUNY, New York, NY 10029 and Department of Neurology, Hackensack Medical Center, Hackensack, NJ 07601.

Imidazoleacetic acid (IAA), which we demonstrated in rat brain and in human CSF [J. Neurochem. 52: 1107, 1989], stimulates GABA-A receptors, produces analgesia, hypnosis, hypotension and other central effects. We found [Soc. Neurosci. Abstr. 15: 998, 1989] high concentrations of an acid-hydrolyzable conjugate(s) of IAA in brain (> 20 nmol/g) and CSF, whose levels exceeded those of free IAA by up to 100-fold. We postulated that the conjugate(s) may have been IAA-ribotide (IAA-Rt) and/or IAA-riboside (IAA-Rs).

IAA-Rs HCl, prepared for us by synthesis, was derivatized with trimethylchlorosilane (TMCS) and analyzed by gas chromatography (GC)-mass spectrometry (MS). IAA-Rs-TMS_4 was identified by chemical ionization [MH^+ 548] and electron impact ionization MS. We have extracted IAA-Rs from urine collected (>48 h) from rats multiply treated with IAA-HCl (i.p.) and D-glucose (p.o.). The TMS derivative(s) of IAA-Rs HCl prepared from urine and by synthesis showed identical GC and MS properties. Patients undergoing diagnostic or therapeutic procedures gave informed consent for collection of additional lumbar cerebrospinal fluid (CSF) for research purposes. In some patients, more than 30 ml of CSF was collected sequentially in 1-2 milliliter units. CSF aliquots of ml 4-5, 16-17 and 29-30, derivatized with TMCS, showed the presence of IAA-Rs. Derivatives of the latter showed GC-MS characteristics identical to those of IAA-Rs prepared from urine. This supports our previous postulate that IAA in brain may be ribosylated and suggests that IAA in CNS of humans may be metabolized by the only route of IAA metabolism known to occur in rodents, i.e. formation of IAA-Rt then dephosphorylation to IAA-Rs. [Research supported by grant NS28012 from NINDS.]

541.9

IMMUNOCYTOCHEMICAL DEMONSTRATION OF SYNAPTIC TRANSMITTERS IN THE LATERAL MEDIAL-SUPRAGENICULATE COMPLEX OF THE CAT. S. Onodera*, M. Norita, K. Takeda* R. Eason and T.P. Hicks Dept. of Psychology, UNC-Greensboro, Greensboro, NC 27412-5001

Immunocytochemical studies with several antibodies raised against various suspected synaptic transmitters have been performed at the light microscope level to identify and characterize the distributions of the neurons and axon terminals containing choline acetyltransferase (ChAT), Substance P, GABA, glutamate and aspartate in the lateral medial-suprageniculate nuclear complex (LM-SG) of the cat. Determination of the boundaries of LM-SG was aided by cytoarchitectonic criteria and by superimposition of adjacent sections treated for acetylcholinesterase (AChE).

The pattern of staining of varicosity-containing, ChAT-positive fibers corresponded well with the pattern of AChE staining seen throughout nearly all areas of LM-SG. Small neurons were heavily immunopositive for GABA. Aspartate and glutamate appeared to be present in moderate to low levels, and were found principally in larger neurons. Substance P staining was very weak and appeared to be located within a few small-diameter fibers that also possessed varicosities. These studies are continuing at the E.M. level to assess the likely nature of the transmitters of LM-SG afferents, particularly those from tectal and cortical sources.

Supported by the Human Frontiers in Science Programme.

541.10

THE MEDIAL AMYGDALOID NUCLEUS OF THE SYRIAN HAMSTER CONTAINS TWO DISTINCT POPULATIONS OF TYROSINE HYDROXYLASE-IMMUNOREACTIVE NEURONS. S.E. Asmus, C.R. Neal, Jr. and S.W. Newman, Department of Anatomy and Cell Biology, University of Michigan, Ann Arbor, MI 48109.

The medial nucleus of the amygdala (Me) integrates chemosensory and hormonal signals influencing sexual behavior in male Syrian hamsters (*Mesocricetus auratus*). Only in this species, to date, have dopamine (DA) neurons been described in Me (Vincent, *J. Comp. Neurol.*, '88), although DA is considered to be important in regulating sexual behavior in male rats. To extend Vincent's observations, we used colchicine to enhance tyrosine hydroxylase (TH)-immunostaining in Me. Nine adult males received intraventricular injections of 200 ug of colchicine and were perfused after 48 hours. Forty-um coronal brain sections were processed for peroxidase-antiperoxidase immunocytochemistry using 3 different TH-antibodies: one monoclonal (Incstar) or one of two different polyclonal antisera (Eugene Tech or East-Acres Biol.). Polyclonal antisera generated against DBH or PNMT (Eugene Tech) were also used to test for the presence of these other catecholamine synthetic enzymes. Unlike Vincent's observations, we found more than 100 TH-immunoreactive neurons within two distinct populations, one in the anterior, and the second in the posterodorsal region of Me. Similar staining patterns were obtained with all TH-antisera. Animals not given colchicine (n=2) had only a few TH-immunoreactive cells in Me. Absence of DBH and PNMT-immunolabeling of Me suggested that these TH neurons are dopaminergic. Whether these DA neurons are involved in regulating sexual behavior will be examined in future studies. (Supported by NIH, NS #20629 to SWN).

541.11

A MICRODIALYSIS STUDY OF BRAIN SEROTONIN FOLLOWING FENFLURAMINE AND QUIPAZINE ADMINISTRATION IN THE PIGEON. C. HARROD*, R.D. JOHNSON* AND J.E. BARRETT. Dept. of Psychiatry, Uniformed Services University of the Health Sciences, 4301 Jones Bridge Rd., Bethesda, MD 20814-4799.

Brain microdialysis techniques were used to examine effects of fenfluramine (10 mg/kg) and quipazine (3.0 mg/kg), both administered i.m., on serotonin (5-HT) release in the raphe and hippocampal areas of the pigeon brain. Dialysates were collected from the awake pigeon following earlier stereotaxic implantations of a guide cannula targeted at the respective sites. Both fenfluramine and quipazine increased 5-HT concentrations in both brain regions, with peak effects occurring approximately 60 minutes following drug injection; effects of both drugs lasted for about two hours before returning to near control levels. These studies demonstrated that the microdialysis procedure can be used to study 5-HT neurochemical systems in the awake, behaving pigeon. Supported by DA 02873.

541.12

MONOAMINES IN THE CHICK BRAIN: REGIONAL DISTRIBUTION AND PRESYNAPTIC MODULATION OF RELEASE. M.L. Dubocovich J.A. Siuciak, and S. Iacob*. Dept. Pharmacology. Northwestern Univ. Medical School, Chicago, Il. 60611.

The levels of monoamines (DA, NE, and 5HT) and their metabolites (DOPAC, MHPG, and 5HIAA) were measured in the brains of one week old chicks which were dissected into the following areas: hypothalmus (HYPO), thalamus (THAL), neo-striatum/ectostriatum (NEO/ECTO); hyperstriatum (HS), paraolfactory lobe/nucleus basalis area (LPO/NB), optic tectum (OT), cerebellum (CER), spinal cord (SC), and pineal (PIN) and assayed using the Coulochem Electrode Array System of ESA Inc. (Bedford, MA.). In the NEO/ECTO, LPO/NB, THAL and HYPO the levels of DA and DOPAC (pg/mg tissue) were: 2 and 16, 92 and 227, 5 and 34, 108 and 99; NE & MHPG were: 26 and 81; 80 and 32, 58 and 489, 526 and 41; and 5HT and 5HIAA were: 13 and 165, 75 and 304, 19 and 352, 206 and 306, respectively. The calcium-dependent release of ^{3}H-5HT from NEO/ECTO slices is inhibited by 5-OCH_3-tryptophan (1-1000 nM) and 8-OHDPAT (1-1000 nM) and is increased by methiothepin (1-1000 nM), suggesting modulation of release by 5HT autoreceptors. In addition the release of ^{3}H-DA is modulated through D_2 autoreceptors. The complete regional distribution of monoamine levels and release modulating presynaptic receptors will be presented. We conclude that in the chicken brain, as in mammals, presynaptic receptors modulate the release of monoamines. Supported by MH42922 to MLD and NS07140 to JAS.

541.13

QUANTATITIVE PHARMACOLOGICAL ANALYSIS OF MELATONIN BINDING SITES IN DISCRETE AREAS OF THE CHICKEN BRAIN. J. A. Siuciak[1], D. Krause[2], M. L. Dubocovich[1], Dept. Pharmacol., Northwestern Univ. Medical School, Chicago, Il. 60611 and [2]Univ. Calif., Medical College, Irvine, CA. 92717.

Melatonin binding sites are widely distributed throughout the chicken brain, predominantly in visual areas. The density and pharmacological characteristics of 2-[^{125}]-iodomelatonin binding sites were determined using quantitative autoradiography. Scatchard analysis of saturation data in representative areas of the chicken brain revealed saturable high affinity binding sites [K_d (pM); B_{max}, (fmol/mg protein)] in the optic tectum (35; 11.4), neostriatum (20; 10), suprachiasmatic nucleus (10; 6.9), and N. rotundus (22; 11.2). *Melatonin and 6-Cl-melatonin* were potent competitors of 2-[^{125}I]-iodomelatonin binding [K_i (pM)], (OT: 62 and 117; NEO: 8 and 63; vSCN: 19 and 47; and ROT: 57 and 68, respectively), while N-acetylserotonin and luzindole show lower affinity [K^i (nM)] (OT: 95 and 633; NEO: 307 and 188; vSCN: 292 and 491; and ROT: 56 and 149, respectively). A complete regional distribution of density and binding affinities for 2-[^{125}I]-iodomelatonin binding sites will be presented. We conclude that high affinity 2-[^{125}I]-iodomelatonin binding sites, with characteristics of ML-1 sites, are heterogeneously distributed through the chicken brain. Supported by MH42922 (MLD) and NS07140 (JAS).

NEUROENDOCRINE REGULATION: OTHER III

542.1

ESTROGEN RECEPTOR GENE EXPRESSION IN THE RAT HIPPOCAMPUS. A. Maggi and E. Bettini*. Milano Molecular Pharmacology Lab, Inst. Pharmacol. Sci., Univ. Milano, Italy.

Recent studies performed in rat hippocampus indicate the presence of relatively high amounts of a protein which is indistinguishable from estrogen receptor (ER) on the bases of electrophoretic, immunological and pharmacological criteria.

In order to fully prove the existence of estrogen receptor synthesis in this important limbic area, the presence of ER mRNA was tested by means of the polymerase chain reaction (PCR) technique. A sscDNA was therefore prepared by oligo dT priming of mRNA extracted from the hippocampus of ovariectomized rats and a series of oligonucleotides were utilized in order to amplify selected portions of the coding sequence. This analysis proved the presence of ER mRNA in rat hippocampus. Sequencing of the amplified section of ER cDNA permitted to determine that the mRNA present in the hippocampus is identical to that one synthesized in uterus.

Further studies are presently being carried on in order to obtain a correct extimate of the concentration of ER in the rat hippocampus.

542.2

SHORT-TERM ELEVATION IN GLUCOCORTICOID LEVELS IS SUFFICIENT TO ELEVATE ADRENAL MEDULLARY PNMT LEVELS P. Boksa, K. Betito, and J. Diorio*. Douglas Hospital Research Ctr., Depts. of Psychiatry & Pharmacology, McGill University, Montreal, Quebec, H4H 1R3

A well known effect of glucocorticoids in the adrenal medulla is the regulation of phenylethanolamine-N-methyl transferase (PNMT) activity. PNMT catalyzes the conversion of noradrenaline to adrenaline in chromaffin cells of the adrenal medulla. Two days of glucocorticoid (GC) treatment has previously been shown to be required for a significant elevation of PNMT levels in cultured bovine adrenal chromaffin cells. Since a short (1-2 hour) pulse of GCs is released in response to a single episode of acute stress, these studies examine the effects of treating cultured bovine adrenal medullary cells for a short time with high dose GCs, mimicking the physiological phenomenon, on PNMT activity 2 days later. Doses of 10 and 100 uM hydrocortisone (HC) were used in the treatments, and in all studies no differences were found between the two doses. Similar to previous results by others, continuous 2 day HC treatment increased PNMT levels to 247% of control. Acute HC treatments of 30 minutes or 2 hours elevated PNMT levels assayed 48 hours later to 174% & 190% of control, respectively. The increase in PNMT levels that resulted from a 2 hr pulse of HC persisted for 72 hrs following treatment. Preliminary data suggest that a 2 hr pulse of HC (100 uM) begins to elevate PNMT levels within 12 hours. These results suggest that short-term elevation in GC levels, as seen physiologically after an acute stressor, is sufficient to elevate adrenal PNMT levels. Supported by MRC of Canada and FRSQ.

542.3

ESTROGEN RECEPTOR BINDING IN REGIONS OF THE RAT HYPOTHALAMUS AND PREOPTIC AREA AFTER INHIBITION OF DOPAMINE-ß-HYDROXYLASE. T.J. Brown, J.D. Blaustein, R.B. Hochberg*, and N.J. MacLusky. Div. of Reprod. Sci., Toronto General Hospital, Toronto, ON M5G 1L7, Neurosci. and Behav. Prog., Univ. of Mass., Amherst, MA 01003, and Dept. of OB/GYN, Yale Univ. Sch. of Med., New Haven, CT 06510.

Previous studies have shown that administration of diethyldithiocarbamate (DDC), a dopamine-ß-hydroxylase inhibitor, results in decreased estrogen receptor concentrations in the rodent hypothalamus. To determine if this modulation of receptor content is region-specific, *in vitro* estrogen binding assays were performed on cytosol and cell nuclear extracts of microdissected brain regions from female rats treated with DDC. For cytosol binding comparisons, ovariectomized (OVX) rats were treated with 550 mg DDC/kg b. wt. or the saline vehicle 12 h before sacrifice. For cell nuclear binding comparisons, OVX rats received a maximal dose of estradiol 12 h after DDC and 1 h before sacrifice. No region-specific decreases in estrogen binding were observed in either cytosol or nuclear extracts. To further examine possible region specificity, quantitative autoradiographic analysis of the *in vivo* hypothalamic uptake of an iodinated analog of estradiol, 11ß-methoxy-16α-[125I]iodoestradiol, after DDC treatment was conducted. DDC treatment resulted in higher levels of radioactivity in serum and neural tissues of [125I]MIE2-injected rats, suggesting that DDC slows the clearance of MIE2. These results suggest that modulation of estrogen receptors in the rodent brain by inhibition of catecholamine synthesis is not manifest with any clear regional specificity, and underscore the need to consider possible multifocal sites of action of pharmacological agents used in studying neurotransmitter regulation of steroid hormone receptors.

542.4

INTENSITY OF NEURONAL ESTROGEN RECEPTOR-IMMUNOSTAINING CORRELATES WITH NORADRENERGIC INPUT IN THE ROSTRAL VENTROLATERAL HYPOTHALAMUS OF GUINEA PIGS. M.J. Tetel and J.D. Blaustein. Neuroscience and Behavior Program and the Psychology Department, University of Massachusetts, Amherst, MA 01003.

The noradrenergic (NA) system plays an important role in the regulation of female sexual behavior and gonadotropin release. We have previously reported dopamine-ß-hydroxylase-immunoreactive (DBH-IR) punctate structures in close association with estrogen receptor-immunoreactive (ER-IR) cell bodies and processes throughout the guinea pig hypothalamus. To determine if intensity of ER-immunostaining is influenced by the presence of NA input, the optical densities (OD) of these ER-IR cells were quantified using an imaging system. The rostral ventrolateral hypothalamus, an area containing many ER-IR cells, is sufficient for estradiol priming of progesterone-facilitated lordosis. In this area ER-IR neurons, with DBH-IR punctate structures closely associated with both the soma and processes, stained more darkly (OD=3.3) than neurons lacking this association (OD=2.6). However, in this experiment the α-NA antagonist, prazosin, did not result in a detectable decrease in the intensity of ER-immunostaining. Nevertheless, the initial finding suggests indirectly that the NA system may maintain the concentration of ERs transneuronally, and it supports the results of previous experiments showing modulation of ERs by the NA system.

(Supported by NIH NS 19327 and RCDA NS 00970)

542.5

EVENING MELATONIN INJECTIONS INHIBIT TUBEROINFUNDIBULAR DOPAMINE SYNTHESIS IN OVARIECTOMIZED HAMSTERS. N. A. Alexiuk and J. Vriend., Dept. of Anatomy, Univ. of Manitoba, MB, Canada R3E 0W3.

In the present study the effects of melatonin administration on the accumulation of serotonin (5HT) and dopamine (DA) after pargyline administration was studied in tissue punches of median eminence and of caudate n. of intact and ovariectomized Syrian hamsters. Concentrations of monoamines and metabolites were determined by HPLC with electrochemical detection. In median eminence, but not in striatum, the accumulaton of DA was reduced to 22% of controls in intact hamsters ($p < .05$) and to 9% of controls in ovariectomized hamsters ($p < .01$) by melatonin injections. Ovariectomy resulted in a significant increase in DA accumulation after pargyline in saline-injected, but not in melatonin -injected hamsters. No significant effects on the accumulation of 5HT in median eminence or in striatum could be detected. Increased levels of 5HIAA in hamsters not receving pargyline were observed both in median eminence and in caudate n. as a result of melatonin injections. These data show that evening melatonin injections inhibit daytime DA synthesis. The results suggest that melatonin may influence the activity of tyrosine hyroxylase in median eminence directly, or indirectly via serotonergic neurons which synapse on tyrosine hydroxylase containing neurons in the median eminence. (Supported by MRC).

542.6

AUTORADIOGRAPHIC LABELING OF NEURONS IN THE BRAINS OF SHORT AND LONG-TERM CASTRATED ADULT MALE RHESUS MONKEYS AFTER ADMINISTERING [^{3}H]TESTOSTERONE. A.N. Clancy, R.W. Bonsall and R.P. Michael, Department of Psychiatry, Emory University School of Medicine, Atlanta, Georgia 30322 and the Georgia Mental Health Institute, Atlanta, Georgia 30306.

In long-, but not short-term, castrated male macaques, testosterone administration does not suppress plasma gonadotropin levels or always fully reinstate copulatory behavior. To examine the proposition that long-term castration alters the uptake of androgens by the brain, 4 adult males castrated 3 days previously (BW 8.8-10.7 kg) and four adult males castrated more than 3 years previously (BW 7.2-10.9 kg) were administered 5 mCi [^{3}H]testosterone i.v. After 60 min, brains were rapidly removed and frozen for autoradiography. Sections were cut in a cryostat at 4 μm, thaw-mounted on emulsion-coated slides and exposed for 20-40 weeks. A total of 8,800 neurons in 11 brain regions were examined using a computerized grain-counting system (Bioquant Meg IV, RM Biometrics), and labeled neurons were identified using a rigorous criterion based on the Poisson distribution. In both short- and long-term castrates, there were significant regional differences in the percentages of labeled neurons ($P<0.001$). Highest percentages (50%-80%) occurred in the ventromedial and anterior hypothalamic nuclei, cortical and basal accessory amygdaloid nuclei, intercalated mammillary and premammillary nuclei, bed nucleus of the stria terminalis (BST), and medial preoptic area. Lower percentages (20-50%) occurred in the lateral septum, arcuate nucleus, and medial amygdala. However, there were no differences in the percentages of labeled neurons between short- and long-term castrates with the sole exception of the BST. Data derived from computerized grain-counting correlated highly ($r=0.8$, $P<0.0001$) with those derived from manual counting methods (USPHS grant MH 19506).

542.7

EFFECTS OF TESTOSTERONE PROPIONATE ON THE DENSITIES OF 5-HT1A AND 5-HT1B RECEPTORS IN THE BRAINS OF CASTRATED MALE RATS. S.D. Mendelson and B.S. McEwen. Laboratory of Neuroendocrinology, Rockefeller Univ. New York, NY 10021

Gonadal hormones modulate the density of 5-HT1 receptors in the male rat brain. However, it has not been known what effects the hormones might have on the subtypes of 5-HT1 receptors in male rats. Effects of activation of 5-HT1A and 5-HT1B receptors on testosterone-dependent sexual behavior have suggested that these receptors may be under gonadal hormone control. Accordingly, adult male rats were castrated under anesthesia and administered either testosterone propionate (TP, 0.2 mg) or the oil vehicle in 14 daily injections (n=7). Labellings of 5-HT1A and 5-HT1B receptors for autoradiographic analyses were accomplished by incubation of brain sections in tris-buffered solutions of 1.5 nM [3H]8-hydroxy-(di-n-propylamino)tetralin or 50 pM [125I]cyanopindolol plus isoproteronol, respectively. In each case, unlabelled serotonin was used to determine non-specific binding. TP produced significant increases in the density of 5-HT1A receptors in the medial preoptic nucleus (MPN), but had no effects on these receptors in areas that included the ventromedial hypothalamus, septum and cortex. TP had no effects on 5-HT1B receptors in any area measured, indicating that testosterone can have differential effects on the subtypes of 5-HT1 receptors. The present data suggest that 5-HT1A receptors in the MPN may mediate facilitation of male sexual behavior in the rat.

542.8

VAGINAL ORIFICE REFLEX DILATION IN INTACT AND OVARIECTOMIZED RATS IS MODULATED BY ESTROGEN AND PROGESTERONE. M. Martínez-Gómez, R. Chirino*, P. Carrillo*, C. Beyer* and P. Pacheco. CIRA-Univ. Aut. Tlaxcala-CINVESTAV, Tlaxcala, México; IIB-UNAM, México, DF; CIB-Univ. Veracruzana, Xalapa,Méx.

Lordosis in estrous rats is often associated with vaginal orifice dilation (VOD). This response is under control of pudendal nerve since electrical stimulation of this nerve induces ipsilateral displacement of the VO in anesthetized rats. Under unstimulated condition the VO is normally closed. Perineal tapping elicits a reflex dilation of the VO (VORD) which is facilitated by ovarian secretion since it is maximal (75%) during estrous and minimal during diestrous (7%). The effect of sex steroids treatment on VORD was studied in ovariectomized rats. Perineal tapping induced VORD only in 21% of ovariectomized nontreated subjects (SS). Treatment with 10ug of EB sc significantly increased the proportion of SS responding to perineal tapping (46%). On the other hand, maximal facilitation of the response occurs in those SS receiving estrogen 10ug plus progesterone 1mg, since 75% of these SS presented VORD by perineal tapping. The present results indicate that VOD is a reflex response that can be induced by perineal tapping in intact proestrous and estrous rats. This response is maximally stimulated in ovariectomized rats by the sequential administration of estrogen and progesterone which normally facilitate lordosis behavior in the rat. The results suggest that under normal conditions fluctuations in sex steroids modulate the responsiveness of pudendal motoneurons to sensory stimulation of the perineal area normally occurring during copulation.

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542.9

GONADECTOMY INCREASES GLUCOCORTICOID CYTOSOLIC RECEPTOR (GCCR) NUMBER IN THYMUS BUT NOT SPLEEN OF ADULT RATS. **R.E. Landsman, A.N. Taylor, R.A. Gorski, B.J. Branch*, J.E. Shyrne*, L.A. Nguyen* and F. Chiappelli. Lab. of Neuroendocrinol., Brain Res. Inst., & Psychoneuroimmunol. Prog., UCLA, & West L.A. VAMC, Brentwood Div., Los Angeles, CA 90024.**

Gonadal hormone-dependent and -independent sex differences exist in GCCR binding sites in rat brain (Turner & Weaver, 1985). Because neuronal and lymphoid GCCRs exhibit some similarities (Lowy, 1989), we examined sex differences and hormone sensitivity in thymus and spleen weight and thymocyte and splenocyte GCCR sites/cell in 80-100 day old intact and gonadectomized (GNX) Sprague Dawley rats. There were no sex differences in spleen or thymus weight for either intact or GNX rats. GNX increased thymus weight 1.6-fold in males ($p<0.004$) and females ($p<0.01$), but had no effect on spleen weight. No sex differences occurred in GCCR sites/cell (using 64 nM [^{3}H]triamcinolone) in either organ for intact or GNX rats. GNX increased GCCR number in male thymus (2.4-fold, $p<0.01$), but not in females. Splenocyte GCCR number was not affected by GNX. Our data indicate that thymic, but not splenic, weight and glucocorticoid receptor number are depressed by gonadal hormones in the rat, suggesting that GCCR may provide a possible mechanism for gonadal hormone modulation of the effects of stress on immune function. **(NIH HD07228 & HD01182, Bettingen Fdn. & VA Medical Res.)**

542.10

EFFECTS OF ESTROGEN ON EXCITABILITY AND DYE COUPLING INCIDENCE AMONG RAT SUPAROPTIC NUCLEUS (SON) NEURONS. Q.Z. Yang and G.I. Hatton. Neuroscience Program, Michigan State University, East Lansing, MI 48824.

Castration in males results in decreased incidence of dye coupling among magnocellular lateral paraventricular neurons, an effect that is reversed by testosterone. In intact males there is more coupling than in intact females among SON neurons, suggesting a possible steroid-dependent sex difference. Effects of estrogen (E_2) removal via ovariectomy (Ovex) were studied in horizontally cut slices from 11 ovex and 11 sham ovex rats. Lucifer Yellow-filled electrodes were used to impale and inject SON neurons. 11 injections in sham ovex slices resulted in 10 single and 2 coupled, dye-filled neurons, whereas 11 injections in ovex slices yielded 6 single and 11 coupled, dye-filled neurons, $\chi^2=4.76$, $p<0.03$. An attempt to reverse the effects of ovex by addition of E_2 (10^{-12}M) to the medium yielded an intermediate amount of coupling, not significantly different from either of the other groups. E_2 added to the medium did augment excitatory responses to micropressure applications of glutamate in some neurons; in others E_2 appeared to interfere with glutamate excitation. We conclude that removal of E_2 has an enhancing effect on interneuronal coupling, opposite in direction to the effects of testosterone removal. Also, E_2 may modulate the effects of glutamate via a direct action on neuronal membranes.

542.11

KYNURENIC ACID BLOCKS EXCITATORY SYNAPTIC RESPONSES OF RAT SUPRAOPTIC NUCLEUS (SON) NEURONS TO LATERAL OLFACTORY TRACT (LOT) STIMULATION. K. G. Smithson & G. I. Hatton Neuroscience Program & Physiology Dept., College of Osteopathic Medicine, Michigan State University, East Lansing, MI 48824

The main (Smithson, K. G. et al. *Neuroscience* 31:277, 1989) and accessory (Smithson, K. G. et al. *Anat. Rec.* 220:91A, 1988) olfactory bulbs project to the SON. Intracellular electrophysiological analysis of this connection in brain slices revealed predominantly short-latency excitatory responses to electrical stimulation of the LOT (Hatton, G. I. & Yang, Q. Z. *Neuroscience* 31:289,1989). Since much evidence suggests that mitral cells of the olfactory bulbs employ excitatory amino acids (EAA) as neurotransmitters, we investigated the possibility that these short-latency responses observed in SON neurons were mediated through an EAA receptor. Using an explant preparation that contained virtually the complete olfactory projection from the rostral end of the LOT to the SON, we examined the effects of bath application of 1 mM kynurenic acid (KYN) on the evoked responses in SON neurons from LOT stimulation. Intracellular recordings were obtained from 66 SON neurons, of these, 50 neurons responded to electrical stimulation of the LOT. The responses observed were all excitatory and had variable latencies. In 15 neurons in which long-term stable impalements were maintained, KYN blocked LOT-evoked responses. In all cases, action potentials could be evoked during the KYN-blockade by intracellular current injection , or when previously possible, antidromically by neural stalk stimulation. These results confirm previous reports of the excitatory nature of this connection. An additional finding in the present study is that these excitatory responses are blocked by KYN a specific antagonist of EAA receptors, a result supporting the notion that mitral cell neurotransmission is mediated, at least in part, via EAA's. Supported by NS 16942 and by a fellowship from a Medical Scientist Training Program to KGS.

542.12

FURTHER CHARACTERIZATION OF HYPOTHALAMIC β-ENDORPHIN NEURONS AND MODULATION OF OPIOID RESPONSES BY ESTROGEN. M.J. Kelly, M.D. Loose and O.K. Ronnekleiv. Dept. Physiology, Oregon Hlth Sci Univ., Portland, OR 97201.

Intracellular recordings were made from arcuate (ARC) neurons with biocytin-filled electrodes in slices prepared from ovariectomized guinea pigs treated with estradiol or oil. Fifty-seven neurons were identified and immunoreacted for β-endorphin (βEND). Fourteen of these cells were immunopositive for βEND. βEND neurons had membrane characteristics (i.e. RMP: -56 ± 1 mV; R_{in}: 380 ± 67 MΩ; τ: 16.5 ± 4.2 ms) similar to immunonegative neurons and often fired spontaneously (6.1 ± 2.0 Hz). βEND neurons exhibited an instantaneous as well as time-dependent rectification. A subpopulation of βEND neurons (N = 4) exhibited an I_A. The μ-opioid agonist Tyr-D-Ala-Gly-MePhe-Gly-ol (DAGO) induced membrane hyperpolarization (12 ± 2 mV) and decreased the R_{in} (38 ± 4%) of the β-endorphin neurons. This response was similar in non-βEND neurons. In preliminary experiments, the EC_{50} for the effects of DAGO (~0.15 μM) was similar in cells recorded from oil- and estradiol-treated animals. The pA_2 values for naloxone antagonism of the effects of DAGO in both groups were in the range reported for other tissues. Experiments are in progress to determine if estrogen causes any shift in the pA_2 value. Thus, μ-receptors may be autoreceptors on ARC βEND neurons, and this "ultra-short loop" feedback mechanism may be modulated by estrogens. (PHS DA 05158, HD 00718).

542.13

IMMUNOCYTOCHEMICAL LOCALIZATION OF AROMATASE AND ESTROGEN RECEPTORS IN THE BRAIN J. Balthazart, A. Foidart, C. Surlemont*, N. Harada*, C. Leranth, and F. Naftolin, Lab. Biochem, Univ.Liège, B-4020 Liège, Belgium, Mol. Genetics, Fujita-Gakuen Health Univ., Toyoake, Japan and Dept. Obstetrics and Gynecology, Yale Univ., New Haven, CT 06510.

The distributions of aromatase (ARO) and estrogen receptors (ER) were studied in the brain of intact male Japanese quail (*Coturnix c. japonica*) by double label immunocytochemistry using a polyclonal antiserum against human placental ARO (J. Biochem.103: 106,1988) and Abbott's monoclonal antibody H222SPγ against human ER. Abundant aromatase-immunoreactive cells (ARO-ir) were found in the medial preoptic nucleus (POM), in the septal region and in a large cell cluster extending from the dorso-lateral aspect of the ventromedial nucleus to the tuberal region of the hypothalamus. Estrogen receptor-immunoreactive cells (ER-ir) were also found in each of these brain areas but their distribution was much broader and included larger parts of the preoptic, septal and tuberal regions. Only a limited proportion of cells were double-labeled (5-30% in POM, <5% in the septum but more than 50% in the tuber). At the EM level, ARO-ir was limited to certain neurons and filled the entire perikaryon, including presynaptic boutons. There were ARO-ir positive boutons forming synapses with ARO-ir neurons (Abst.669, The Endocrine Society, 1990). CONCLUSIONS: The present study shows that ER-ir is more broadly distributed and only present in some of the ARO-ir cells. These findings indicate that in addition to the usual ER-mediated actions, locally formed brain estrogen may have non-ER-mediated actions including the synaptic level and elsewhere in brain neurons. Supported by NIH HD22064, FNRS and EEC (SC1-0230-C/TT) to JB, NS266068 and NIH HD2383 to CL and NIH HD13587 to FN.

SENSORY SYSTEMS—SUBCORTICAL VISUAL PATHWAYS: MIDBRAIN, ETC.

543.1

THE LAMINAR DISTRIBUTION AND MORPHOLOGY OF CENTRIFUGAL AXONAL TERMINATIONS IN THE PIGEON RETINA (*Columba livia*). W. Woodson, T. Shimizu and H. J. Karten. Dept. of Neurosciences, U.C.S.D., La Jolla, CA 92093.

Following injections of phaseolus vulgaris leucoagglutinin, PHA-L, and rhodamine isothiocyanate into the isthmo-optic nucleus (ION), the laminar distribution and morphology of ION afferent fibers to the retina (centrifugals) were studied in transverse and horizontal sections.

In transverse sections through the retina, while both markers revealed a heavy band of terminals confined to lamina 1b of the inner plexiform layer(IPL), and fibers penetrating into the inner nuclear layer, PHA-L also showed sparse labelling in lamina 5b. Immunocytochemical studies have indicated that 5-HT is also located in laminae 1b and 5b of the IPL.

Horizontal sections through the retina showed that fibers entering the optic nerve head, some of which bifurcated, ranged in diameter from 0.1 to 1 μm. Three types of centrifugal arborizations were observed: Type 1: thin fibers expanding over a large area of the retina, having as many as 70 terminal arborizations, Type 2: thin axons, terminating over a confined surface area, possessing up to 80 terminal endings, and Type 3: thick fibers, having a restricted terminal field with as many as 60 terminal boutons. While there was little variation in the number of terminations for the three categories of fibers, the terminal surface area occupied by type 1 axons was approximately 7 times greater than the other types of axonal terminal endings.

In summary, a large number of amacrine and displaced ganglion cells are influenced by single centrifugal fibers, either over a widely spaced or confined surface area of the retina. Moreover, centrifugals could modulate 5-HT positive amacrine cells, suggesting that the ION modulates various subpopulations of morphologically and biochemically distinct amacrine cells in the retina.

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543.2

POTENTIAL PATHWAYS FOR VISUAL AND ELECTRO-SENSORY INFORMATION TO REACH THE TELENCEPHALON. **E. Sas and L. Maler, Dept. Anatomy, Univ. of Ottawa, 451 Smyth Rd., Ottawa K1H 8M5 Canada.**

This is the first attempt in Apteronotus leptorhynchus, to investigate possible routes for visually related and electrosensory information to reach the pallium. To this end electrophoretic injections of WGA-HRP were placed in the dorsal subdivision of the dorsolateral telencephalon (DLd) which resulted in labeling of the following nuclei: telencephalic areas dorsalis pars magnocellularis, dorsolateralis pars posterior, dorsalis centralis (DC), and the large cells of rostral nucleus entopeduncularis (Er); the diencephalic preglomerular complex pars lateralis (PGl), the mesencephalic nucleus raphe centralis, and in the rhombencephalon, the locus coeruleus and the paramedian medullary reticular formation. Although DLd appears as a homogenous cellular mass in cresyl violet sections, superficial and deep injections into DLd indicated a stratified arrangement of some of its afferents and also some differences in input to rostral versus caudal DLd. An interesting observation was that the region of PGl that receives tectal input, projects to DLd, whereas cells dorsal to these PGl cells, are the target of toral input; these latter cells project to the dorsal telencephalon, therefore providing a route for these two types of sensory information to reach the dorsal pallium via separate populations of the preglomerular complex. Injections into more medial telencephalic regions resulted in labeling dorsal and medial aspects of the preglomerular complex. The densest projection from DLd is to that region of DC which projects to the tectum; therefore visually related information reaching DLd via PGl may be fed back into the tectum.

543.3

THE MEDIAL BASAL OPTIC ROOT IS COMPRISED OF AXONS ORIGINATING FROM GANGLION CELLS IN THE CENTRAL RETINA OF FROGS. Z. Li* and K. V. Fite, Neuroscience and Behavior Program, University of Massachusetts, Amherst, MA 01003.

The nucleus of the basal optic root (nBOR) of anuran amphibians is innervated by the basal optic root (BOR) which includes both medial (BORm) and lateral (BORl) fascicles. BORm innervates the mediodorsal portion of nBOR and 98.5% of its axons are unmyelinated (mean = .27 μm diameter). In contrast, BORl innervates the ventrolateral and central portions of nBOR and contains many more myelinated axons. Unmyelinated axons are of larger caliber (mean = .42 μm) than in BORm (Fite, et al, 1988).

Large, efferent ganglionic neurons occur in the mediodorsal portion of nBOR and project exclusively to the dense-core region of the pretectal nucleus lentiformis mesencephali (nLM). The dense core of nLM receives optic afferents from the central portion of the contralateral retina and contains large neurons which project to the optic tectum (Montgomery, et al, 1985).

Localized HRP injections of mediodorsal nBOR retrogradely labelled axons only in BORm and small ganglion cells in the central one-third of the contralateral retina. Thus, the projection from nBOR to nLM may also convey information from the central retina, with specific effects upon the dense core and large neurons of nLM and its function in oculomotor reflexes and optokinetic nystagmus (Supported by NSF grant: BNS 8819870).

543.4

GAD-LIKE IMMUNOREACTIVITY IN CENTRAL VISUAL AREAS OF RANA PIPIENS. C. J. Tyler, K. V. Fite, and G. J. DeVries, Neuroscience and Behavior Program, University of Massachusetts, Amherst, MA 01003

Immunocytochemistry was used to characterize the presence of glutamic acid decarboxylase (GAD), the synthesizing enzyme for gamma-aminobutyric acid (GABA), in primary retinal terminal fields and thalamic nuclear groups postsynaptic to primary retinal terminal zones.

Preliminary observations revealed GAD-like immunoreactivity in all primary visual nuclei. Dense GAD-like immunoreactive (GAD-LIR) puncta were observed in the posterior thalamic nucleus, uncinate nucleus, and nucleus lentiformis mesencephali (nLM). In addition, GAD-LIR perikarya were observed positioned along the lateral and ventral margins of nLM. Present evidence suggests the presence of GAD-LIR terminals in all lamina of the optic tectum, with GAD-LIR perikarya appearing most noticeably in the 6th and 8th lamina. The lateral margins of the basal optic root nucleus contained scarce populations of GAD-LIR perikarya which surrounded the GAD-LIR puncta within the nucleus itself. Both the corpus geniculatum and nucleus Bellonci contained GAD-LIR fibers and puncta. Magnocellular neurons in the nucleus profundus also showed dense GAD-LIR perikarya and dendritic morphology.

In the thalamus, the ventrolateral area, posterocentral nucleus, and posterolateral nucleus (cell groups in contact with retinal afferents) contained both GAD-LIR perikarya and puncta.

Comparisons between GAD- and GABA-like immunoreactivities in these areas will also be presented. (Supported by NSF Grant BNS 5-25576 to K.V.F.)

543.5

EVIDENCE FOR DIRECTION-SENSITIVE (DS) RETINAL INPUT TO THE TURTLE'S BASAL OPTIC NUCLEUS (BON). A.F. Rosenberg and M. Ariel, *Dept. of Behavioral Neuroscience, University of Pittsburgh, Pgh., PA 15217.*

Turtle BON cells respond best to global image motion in a preferred direction (Rosenberg & Ariel 1990), consistent with their purported role in encoding retinal slip. The DS character of BON cells is thought to be signalled by DS retinal ganglion cells (RGC) via the direct contralateral retinal input, based on evidence that BON cells are (1) DS in the absence of the telencephalon, but (2) not DS during retinal bicuculline application (Schuerger et al. 1990).

To test whether DS RGCs project directly to the BON, RGCs were characterized during single unit recordings and then were tested for antidromic activation by electrically stimulating the BON. The preparation was an *in vitro* whole turtle brain, with the eyes attached and telencephalon removed. RGCs were characterized based on their response to steps of diffuse illumination and moving whole-field checkerboard patterns. Current passed through a bipolar electrode was used to stimulate the BON.

Of 34 RGCs recorded, 5 could be activated antidromically using current < 200 μA. Four of these were DS. Antidromic spikes had latencies ranging from 1.6 to 3.8 ms, and could follow stimulation trains exceeding 100 Hz. These latencies were shorter and less variable than those of orthodromic spikes (2.5 - 7 ms, n = 15) recorded from BON cells, elicited by contralateral optic nerve stimulation. Orthodromic spikes recorded in the BON failed to be evoked by consecutive current pulses delivered to the optic nerve at stimulation rates > 30 Hz. Based on these measurements, the direct retinal input to the BON is moderate to rapidly conducting, as suggested by Woodbury and Ulinski (1986). This antidromic activation of DS RGCs is direct evidence for retinal processing providing direction-sensitivity to BON neurons. (Supported by EY05978)

543.6

FUNCTIONAL ORGANIZATIONS IN THE NUCLEUS ROTUNDUS OF PIGEON. Y-C. Wang and B.J. Frost, Departments of Physiology and Psychology, Queen's University, Kingston, Ontario, Canada, K7L 3N6.

Anatomical studies have shown that different rotundal zones receive input from different tectal laminae and in turn project to different areas of ectostriatum. Our previous results indicated that there were at least 6 different types of stimulus specific cells in the nucleus rotundus (Rt), and in this study on Ketamine/Rompun anestheized pigeons, we report correlations between these functional classes and their location within Rt. We have found that most luminance units were located in ventral zones of the nucleus. These units had very large receptive fields (100-140 degrees on average) and either systematically increased or decreased their responses to increases in luminance of the whole visual field. In contrast, most loom units were found in the dorsal-medial part of the Rt. These units respond to Z-axis movement, that is expanding or contracting stimulus patterns, and exhibited neither X-Y directional selectivity nor "on-off" responses. Occlusion sensitive units, of both the excitatory and inhibitory type, were mostly located in posterior Rt zones which receive input from deep tectum. We have also found that although some loom and occlusion units decrease their response to changing wavelengths, most of them still give reliable responses to equiluminous chromatic stimulus movement. Supported by NSERC grant A0353 to BJF.

543.7

DISTINGUISHING ROTATION FROM TRANSLATION: NEURONS IN PIGEON VESTIBULOCEREBELLUM SPECIFY DIFFERENT PATTERNS OF WHOLEFIELD MOTION: D.R. Wylie and B.J. Frost. Dept. of Psychology, Queen's University, Kingston, Ontario, Canada, K7L 3N6.

Several studies demonstrate that the Accessory Optic System (AOS) is involved in the analysis of wholefield visual motion which results from self-motion of an animal. Electrophysiological studies have found that neurons in the pigeon AOS respond best to wholefield visual stimuli moving in a particular direction in the contralateral visual field, however, few cells are binocular. By combining information from both eyes, translation can be distinguished from rotation. Neurons preferring the same or opposite directions in the two eyes would encode translation and rotation respectively. The pigeon vestibulocerebellum (VbC) receives bilateral input from primary AOS structures directly and via the inferior olive, thus there is the potential for binocular interactions. It was the purpose of this study to investigate the responses of neurons in the pigeon VbC to wholefield visual motion. Pigeons were anaesthetized with urethane and extracellular recordings were made with glass covered tungsten microelectrodes. Complex spike activity of Purkinje cells and presumed climbing fibre potentials isolated outside the Purkinje layer responded to wholefield motion. Three functional types were found: neurons preferring upward motion in both eyes, which would result from downward translation; neurons preferring downward motion in both eyes, which would result from upward translation; and neurons preferring backward and forward motion in the contralateral eye and ipsilateral eyes respectively, which would result from a horizontal head or body rotation. Binocular wholefield motion of the preferred direction in both eyes always resulted in a facilitation of the response relative to the dominant eye. A few presumed granule cells responded to monocular wholefield motion. We conclude that neurons in the VbC integrate wholefield information from the two eyes so that self-produced translation can be distinguished from rotation.

543.8

IMMUNOHISTOCHEMICAL PARCELLATION OF THE VENTRAL LATERAL GENICULATE NUCLEUS & INTERGENICULATE LEAFLET IN THE GROUND SQUIRREL (Citellus tridecemlineatus).
S. AGARWALA*, M.A. BASSO*, J.G. MAY , J.K. MOORE, & H.M. PETRY. Depts. of Psychology and Anatomical Sciences, SUNY at Stony Brook, NY 11794.

The ventral lateral geniculate nucleus (vLGN) is well developed in the ground squirrel and may be differentiated into four divisions (the inter-geniculate leaflet (IGL), dorsal cap (DC), external magnocellular (vLGNe), and internal parvocellular divisions) based on it's connectional anatomy and cytoarchitecture (Agarwala et al., 1989). The immunohistochemical signature of these subdivisions and that of the subgeniculate nucleus (SGN) was evaluated with Neuropeptide Y (NPY), Leu-Enkephalin (L-Enk), Cytochrome Oxidase (CO) and Acetylcholinesterase (ACHE). NPY+ cells were found in the external portion of the vLGNe, the IGL, and along the ventral and lateral margin of the DC. These cells formed a shell around the DC which was itself free of NPY+ cells. In contrast to the IGL, the DC and vLGNe were highly reactive for CO and ACHE. Preliminary results from L-Enk correspond to the NPY pattern, (i.e, heavy staining in IGL, faint staining in DC and vLGNe). The SGN was highly reactive for NPY, L-Enk, CO, and ACHE. These immuno- and histochemical results suggest that a functional differentiation may coincide with the anatomical divisions of the vLGN and SGN. Interestingly, this pattern differs from that of the rat and hamster.
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543.9

ULTRASTRUCTURAL CHARACTERISTICS OF THE MEDIAL PRETECTAL NUCLEUS IN THE CAT. R.Pisana*, A.D.Pearsall, and B. Hutchins. Baylor College of Dentistry, Dallas,Tx. 75246

The present study was undertaken to analyze the ultrastructural characteristics of the medial pretectal nucleus, located at the meso-diencephalic junction. According to the basic terminology of Guillery, ('69), the following terminals were identified within the nucleus, RLD, RSD, RLP, PD1,and PD2. All terminals were found to be extraglomerular. The predominant terminal was of the presumptive dendritic type, the PD2, which were primarily found to contact medium sized dendrites. RLD terminals, which have been identified as afferent terminals to the pretectal complex [eg, visual cortex, Hutchins and Weber, Anat. Rec. 205 ('83); Neurosci. Abst. 19 ('89); superior colliculus, Lieberman, et al., Neurosci. Lett. 56 ('85)], were observed to contact small to medium size profiles. Fewer profiles were of the presumptive retinal terminal type, the RLP, which were primarily found contacting medium sized dendrites. In order to identify retinal terminals, HRP was injected in one eye, the tissue was reacted with TMB, and then replaced with DAB for electron microscopy. In this tissue, a few small terminals were labelled with HRP. Thus, this study provides preliminary data demonstrating direct retinal input to the medial pretectal nucleus in the cat.

Supported by NIH grant EY06977.

543.10

THE VISUAL ORGANIZATION OF THE POSTERIOR PRETECTAL NUCLEUS IN THE CAT. B.Hutchins and R.Pisana*. Baylor College of Dentistry, Dallas, Tx. 75246.

The pretectal complex is located at the meso-diencephalic junction and is composed of five separate nuclei. After tissue is processed for either an HRP or a ^{3}H-proline injection into one eye of an adult cat, the direct retinal projections to the pretectal complex were identified. One little studied pretectal nucleus, the NPP receives a patchy, bilateral input. These patches appear to follow the dorsal posterior border and extend the entire medial to lateral extent of the nucleus. This leaves the majority of NPP without identifiable retinal projections. To test whether the remaining portions of NPP are visually related, the nucleus was sampled electrophysiologically in paralyzed chloralose anesthetized cats. Data collected thus far, indicated the full extent of NPP is visually responsive. Representations of the periphery were identified along the NPP/superior colliculus border, while representations of the vertical meridian were identified more anterior and the lower visual field was located lateral. In addition, approximately one half of the extracellular tissue sampled responded to auditory stimuli..Thus, these preliminary data indicate a more complex role for NPP in integrating multisensory information.

Supported by NIH grant EY06977.

CONTROL OF POSTURE AND MOVEMENT: CLINICAL STUDIES

544.1

ARE THE ABNORMAL MOVEMENTS OF TARDIVE DYSKINESIA COMPLETELY RANDOM? JB Lohr, L Ing*, and MP Caligiuri. Motor Function Laboratory, VA Medical Center, V-116A, San Diego CA 92161.

One of the clinical hallmarks of choreoathetoid disorders is their spatial irregularity. In the case of tardive dyskinesia, however, questions have been raised as to how truly random the movements are. In an effort to address this question, we attached a biaxial accelerometer to the tip of the thumb of the more dyskinetic hand in patients with TD of varying degrees of severity as determined by the AIMS upper extremity score. Data were analyzed by plotting the acceleration values associated with the abnormal movements in cartesian coordinates. We then quantified the proportion of activity associated with a particular acceleration vector, yielding an r value, which is a measure of the spatial irregularity. Results showed that greater r values were associated with more severe TD, indicating that with increasing severity of the dyskinesia, the movements were more irregular. Therefore, the movements of patients with milder TD are not completely random, but do show spatial regularity. These results will be contrasted with similar measurements made in patients with Huntington's disease, the prototypic choreoathetoid disorder.

544.2

DIMENSIONALITY OF POSTURAL STEADINESS. JB Myklebust, TE Prieto*, BM Myklebust, DG Lange. Marquette University, College of Engineering, Laboratory of Sensory-Motor Performance,VA Medical Center, Medical College of Wisconsin, Milwaukee, WI 53295 and Sec Computers & Insts NIH-NINDS, Bethesda, MD.

Deviations of the center of pressure (CP) during quiet standing have been characterized for healthy adults. These measurements have been used to assess neurological disorders of vestibular and cerebellar systems, and lesions of pyramidal and extrapyramidal tracts. In previous studies[1] the fractal dimension of the planar curve was used to analyze balance data. The algorithm was developed for segmental curves and may be a simple, practical method to assess standing balance and characterize pathology. In the present study, the fractal dimension, computed using the "coastline method", and a box counting method were compared to the previous algorithm. The slope of the frequency spectrum was a power law function. For comparison, the time series was embedded in higher dimensions and the correlation dimension computed[2].

Data was used from 5 normal young adults (age<40), 5 healthy aging subjects (age 55-65), and 5 elderly patients with Alzheimer's disease with clinical impairments of gait and balance. Standing balance trials were sampled at 100Hz for 1 minute with eyes open and 1 minute with eyes closed. The CP was analyzed for each trial. The fractal dimension obtained by the coastline and box counting methods did not differ significantly from the value computed using the previous algorithm. The mean value tested with eyes open was 1.97 (sd=0.22) for young subjects, 1.73 (sd=0.06) for healthy aging subjects and 1.58 (sd=0.09) for Alzheimer's patients. With eyes closed, the normal subjects did not change; the Alzheimer's group improved to 1.79 (sd=0.05). The slope of the power spectrum was -2.5 for young adults, -3.0 for healthy aging subjects and -3.2 for Alzheimer's patients. The correlation dimension calculated from the embedded time series was lower than the fractal dimension.

1. Myklebust JB,Myklebust BM: Fractals in Kinesiology, Soc Neurosci Abs 15,#243.2,p.604,1989. 2. Mayer-Kress G. ed: Dimensions and Entropies in Chaotic Systems. Springer-Verlag, N.Y., 1985.

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

544.3

Evidence for Separable Effects of Alzheimer's and Parkinson's Diseases on Movement Preparation and Execution Processes. PC Amrhein, Dept. of Psychology, Univ. of New Mexico, Albuquerque, NM 87131, and JC Morris, Dept. of Neurology, Washington Univ. Sch. of Med., St. Louis, MO 63110.

A study was conducted to determine the effects of Alzheimer's Disease (AD) and Parkinson's Disease (PD) on cognitive/motor processes underlying motor plan preparation and execution. Using a 2 [AD: Absent or Present] X 2 [PD: Absent or Present (unmedicated)] design, four subject groups: 10 control subjects; mean age = 71.1 yrs), 6 AD subjects; (mean age = 73.1 yrs, very mild dementia), 11 PD subjects (mean age = 70.6 yrs, no dementia), and 5 AD/PD subjects (mean age = 69 yrs, very mild dementia) were assessed with respect to upper extremity movement for reaction time (RT) and movement time (MT). A paradigm was used in which 75% of the trials had valid precue stimuli (i.e., identical to the target stimuli) but the remaining 25% had invalid precue stimuli (i.e., different from the target stimuli), thus necessitating restructuring of the prepared response. Subjects responded to one of four target stimuli by pressing a corresponding button. Group differences concern overall RT and MT: AD, but not PD, increased RT [$F(1,28) = 4.11, p = .05$ and $F < 1$, respectively]. However, AD and PD exhibited additive effects in increasing MT [$F(1,28) = 6.08, p < .025$ and $F(1,28) = 13.7, p < .001$, respectively]. Results indicate that AD (at a very mild level of dementia) and PD independently influence cognitive/motor task performance: AD, but not PD, slows motor plan preparation (RT); however, AD and PD each slow motor plan execution (MT).

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544.4

MOTOR LEARNING IN PARKINSON'S DISEASE: SCHEMA FORMATION, ADAPTATION TO ALTERED GAIN, AND LIMB KINEMATICS. C.J. Worringham, C.L. Cross* and A.L. Smiley-Oyen.* Department of Kinesiology, The University of Michigan, Ann Arbor, MI 48109.

Motor learning in Parkinson's disease (PD) subjects was studied in a task requiring the learning of an arbitrary relationship between the length of visually-presented horizontal bars and the amplitude of linear, horizontal arm movements. Four targets were initially learned with error feedback. Learning and extrapolation were assessed using no-feedback trials to, respectively, the original targets and targets beyond the range of those previously practiced. A final phase required adaptation to an altered gain. PDs were capable of learning the initial task, and, along with controls, exhibited a range effect (short and long targets over- and undershot, respectively). On extrapolation, the slope of the function relating actual to required movement amplitude fell sharply for PDs, with larger undershoots of longer targets. PDs showed substantial adaptation to a new gain within 144 trials. These results and kinematic data show that the formation and use of higher-level "schema" representations of motor tasks is not lost in PD but in some subjects is degraded.

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544.5

SENSORIMOTOR DISINHIBITION IN PARKINSONISM MP Caligiuri, WC Heindel and JB Lohr. Motor Function Lab. VA Medical Center, San Diego, CA 92161

The inability to execute simultaneous movements is well recognized in Parkinson's disease (PD). Investigators have proposed a number of hypotheses to explain this inability, including deficits of attention, motor programming, or both. In the present study we investigated the role of sensorimotor disinhibition as a mechanism underlying the inability of PD patients to execute simultaneous motor acts. The patients were examined after a 10h drug-free interval. The motor examination required that the subject exert low levels of isometric finger flexion force for 20 seconds under two conditions: (a) with the non-test hand at rest and (b) with the non-test hand engaged in a reaction time (RT) test in which ballistic force pulses served as the response to auditory stimuli. Analyses were made of the degree and pattern of force instability. Results indicated that the PD patients exhibited significantly greater isometric instability than controls. Force instability increased in both control and PD groups during the RT task; however, this increase was significantly greater for the PD than the NC groups. Further analyses revealed that the isometric force waveforms for the PD patients, but not the controls, varied systematically with the RT pulses of the contralateral hand. This pattern of coherence for the two hands may be explained by sensorimotor disinhibition. In PD, the motor commands for executing ballistic muscle force (RT) may be uninhibited and pass through the system programmed for isometric control.

544.6

ABNORMALITIES IN GAIT INITIATION IN PATIENTS WITH PARKINSON'S DISEASE. K. Tako*, R.G.Lee, and W.J. Becker. Dept. of Clinical Neurosciences, University of Calgary, Calgary, Alberta, Canada T2N 4N1.

Patients with Parkinson's disease (PD) often show difficulty in gait initiation. To investigate this further, we studied the timing of movement onset of the trunk and legs during the initiation of walking from the standing position.

Shoulder, hip, knee and foot movements were recorded with a Watsmart position analysis system and EMG was recorded with surface electrodes from the lower leg muscles during the 1st step while patients and controls began to walk in response to an auditory tone.

Preceding toe off of the swing leg, prominent EMG activity occurred in the pretibial muscles bilaterally, the knee of the stance leg began to move forward and then forward movement of the shoulder and hip occurred in both patients and controls. Forward movement of the shoulder and hip followed the initial forward displacement of the stance knee with a shorter time interval in patients than controls. Patients with PD showed a greater delay in gait initiation in response to the auditory tone and a longer time interval between pretibial muscle EMG onset and toe off of the swing leg than controls.

In gait initiation, patients with PD show not only delayed initiation of movement, but also a disordered pattern of leg and trunk movements.

544.7

DURATION OF POSTURAL RESPONSES IN CLUMSY CHILDREN: ANOTHER LOOK AT TIMING CONTROL. H. Williams, S. Jay*, M. Woollacott. Motor Control Laboratory, U of Oregon, Eugene, Oregon 97403.

Skilled movement requires appropriate timing of muscle activity. Children with developmental apraxia are known clinically to have difficulty performing a wide variety of motor tasks & have been shown to have disturbances in both sequencing of postural responses & in timing of rhythmic, repetitive movements of distal extremities.

The purpose of the study was to further examine timing control in clumsy children by studying duration of muscle responses to perturbations of balance. 14 clumsy and 14 normal children stood on a moveable platform; balance was perturbed in randomly ordered trials in forward/backward directions, with/without vision, with modified & normal vestibular input. 3-way repeated measures MANOVAs were used to analyze mean & variability of duration of EMG activity in 8 muscle groups. Duration of muscle activity was longer in clumsy children & was significantly longer with vision than without. With modified vestibular input, both clumsy and normal children showed increased duration & variability of postural responses. These data suggest that clumsy children may have widespread problems with timing of muscle responses integral to movement control.

544.8

EMG CHANGES IN GAIT FOLLOWING TENDO-ACHILLES LENGTHENING IN CEREBRAL PALSY CHILDREN, B. R. Einyre[1], C. S. Chambers[2] and N. H. Scarborough[2]. [1]Human Performance and Health Sciences Dept., Rice University, Houston, TX 77251, [2]Gait Analysis Laboratory, Shriners' Hospitals for Crippled Children, Houston Unit, Houston, TX 77030

Due to spasticity, children with cerebral palsy typically demonstrate an equinus gait from overactivity of the triceps surae muscles. Surgical correction for the equinus gait pattern is done either by lengthening the Achilles tendon or recession of the gastrocnemius aponeurosis. The purpose of this study was to determine if surgery reduced muscle activity and if one surgical method could be considered more effective for reducing muscle overactivity during gait. Twenty-three hemiplegic or diplegic children participated both pre- and post-operatively in a gait analysis laboratory. EMG was collected using surface electrodes applied over the triceps surae muscle of the involved limb or limbs. Percent time of muscle activity during each step cycle was calculated for each subject. Analysis of variance revealed significant reductions of muscle activity during the gait cycle between pre-operative (64.0%) and post-operative (50.4%) measures, $F(1,21) = 47.5$, $p < .001$. No significant difference in EMG for percent of gait cycle between the two surgical methods was observed. It was concluded that either surgical method of Achilles lengthening was effective in reducing the spastic activity in triceps surae muscles of cerebral palsy children. These structural changes were apparently effective in changing either the level of spastic response or the motor program for walking which resulted in a more normal pattern of EMG activity during each gait cycle.

544.9

THE PHYSIOLOGY OF GAIT INITIATION. R.J. Elble, C. Moody* and C. Higgins*. Southern Illinois University School of Medicine, Springfield, IL 62794-9230.

Patients with neurological disturbances of gait frequently have particular difficulty initiating a first step. We therefore undertook a study of gait initiation in eight neurologically normal adults, ages 18 to 87. A quick step forward was taken in response to a green light. The center of pressure of each foot was recorded with floor-mounted force plates, and surface electromyograms were recorded from the tibialis anterior, quadriceps femoris, hamstrings, and gastrocnemius. Motion of the upper and lower extremities and torso was recorded with computerized infrared stroboscopic photography. With a simple reaction time of 0.16 to 0.31 seconds, a forward step was initiated by synchronous contraction of the tibialis anterior and quadriceps femoris. Approximately 0.4 seconds later, synchronous contraction occurred in the gastrocnemius and hamstrings. This stereotypic pattern of muscular activity caused the resultant center of pressure of both feet to move posteriorly toward the swing foot and then ultimately toward the stance foot. These foot-floor reaction forces and muscular activities created forward moments of force about the ankles and hips that propelled the body into forward motion. (Supported by the Whitaker Foundation)

544.10

A COMPARISON IN NEUROLOGICALLY INTACT AND SPINAL CORD INJURED OF ANKLE JOINT COMPLIANCE AND REFLEX ACTIVITY TO ANGULAR PERTURBATIONS USING VOLITIONAL AND ELECTRICALLY STIMULATED BIASES. B. Flaherty,*[1,2] C.J. Robinson,[1,4] G.C. Agarwal[3] & G.L. Gottlieb.[5] Hines VA Rehabilitation R&D Center,[1] Hines, IL 60141; Bioengr.[2] & EECS[3] Depts., Univ. of Illinois @ Chicago; Neurology Dept,[4] Loyola Univ., Maywood, IL; Physiology Dept.,[5] Rush Medical College, Chicago, IL

Ankle compliance characteristics were investigated for complete (n=3) and incomplete (n=1) paraplegics and in 4 intact subjects using a 10° perturbation about neutral ankle angle at various constant velocities (13.3 to 100 °/sec) against a plantarflexed torque bias that was achieved via soleus stimulation. Responses were compared with those obtained via volitional plantarflexed bias in the neurologically intact. Soleus and tibialis anterior surface EMG activity was recorded using an artifact-suppression stimulator/preamplifier. Torque, angular displacement and acceleration were measured and used to calculate stiffness, damping and inertial factors.

During the first 100 msec of a perturbation (any speed), the compliance of the joint was the same under stimulated and volitional biases, indicating that the passive properties of the joint were the predominant influence since no reflex EMG activity was seen until at least 80 msec. After 100 msec, torque continued to be proportional to displacement for the volitional torque bias experiments, while in the stimulated case torque was not linearly related to angle. This was particularly apparent at the end of the perturbation where torque exponentially decayed to a steady state value that was significantly less than the post-perturbation torque under volitional bias. The ankle was also stiffer with voluntary plantarflexion than with soleus stimulation, and the compliance model was more complicated with soleus stimulation.

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544.11

KINETIC ANALYSIS OF INTERLIMB COORDINATION DURING DYNAMIC TRANSITIONS IN STANCE SUPPORT IN ADULT HEMIPARESIS. M.W. Rogers, L.D. Hedman*, and Y.C. Pai*. Physical Therapy, Northwestern Univ. Med. Sch., Chicago, IL 60611.

Dynamic transitions in stance support accompanying intentional leg flexion movements are due in part to the coordinative interaction of the ground reaction forces (GRFs) beneath the upcoming flexing (fl) and single stance (st) limbs prior to and during unloading (UNL) of the fl leg.

The extent to which the spatial and temporal features of the lateral horizontal (Fy) GRF components prior to UNL may be altered by right hemiparesis due to stroke, was investigated in 8 adult subjects who stood on 2 separate force platforms and performed single rapid leg flexion movements with the paretic (PL) and the uninvolved (UL) limbs. The results indicated that the resultant Fy onset always preceded UNL onset (-270 ± 93ms), and was responsible for the linear displacement of the body mass laterally. For UL flexion movements, however, Fyfl contributed a greater ($p<.05$) proportion (89 ± 15%) to Fy than Fyst (11 ± 15%), while PL flexion movements showed a reverse trend ($p<.05$) whereby Fyfl and Fyst became 30 ± 13% and 70 ± 13% of Fy. Strikingly, while Fyfl and Fyst onsets are normally coincident, 4 subjects showed a delay (140 ± 21ms) in PL Fy vs. UL Fy onset times. Moreover, in 2 cases of UL flexion movements Fyst of the PL was exerted in the opposite direction to that of Fyfl of the UL so as to brake rather than assist in the weight transfer to the PL. Overall, such changes in both the timing and scaling of interlimb GRFs may contribute to deficits in lateral weight transfer regardless of the direction of the postural transition.

Supported by the Foundation For Physical Therapy.

544.12

PRESENCE OF RESIDUAL MOTOR UNIT ACTIVITY UNDER VOLITIONAL CONTROL IN THE PARALYZED MUSCLES OF SPINAL CORD INJURY SUBJECTS. W.B. McKay, M.R. Dimitrijevic, M.A. Lissens*, W.A. Nix*. Division of Restorative Neurology and Human Neurobiology, Baylor College of Medicine, Houston, Texas 77030.

We have carried out neurophysiological studies in 297 clinically complete spinal cord injury subjects; in 6 of them we were able to demonstrate the subclinical presence of volitionally controlled single motor units (SMU). Three of these 6 subjects showed the activation of SMU only during particular single-joint motor tasks repetitively and consistently. Therefore, it is possible to demonstrate the preservation of a specific motor command even in subjects who have a limited number of conducting axons reaching their target.

We shall discuss how these reported results are pertinent for the understanding of neurocontrol of an isolated single-joint movement motor task as compared to the patterned, multi-joint motor task.

544.13

FEATURES OF NEUROCONTROL DURING GAIT WITH REDUCED BRAIN INFLUENCE IN HUMANS WITH TRAUMATIC SPINAL CORD INJURY. J.H. Schild*, M.M. Dimitrijevic*, I. Petronic*, A.M. Sherwood. Division of Restorative Neurology and Human Neurobiology, Baylor College of Medicine, Houston, Texas 77030.

We have studied neurocontrol in 13 ambulatory spinal cord injury subjects while they walked on a 10 meter path. We recorded bilaterally and simultaneously during gait with surface electrodes the polyelectromyographic activity of the paraspinal, quadriceps, hamstring, tibialis anterior and triceps surae muscles. In addition, we also recorded gait with foot switches and anterior, posterior and lateral videocameras.

The analysis of data during gait revealed 3 different neurocontrol features: the 1st resembled the neurocontrol in a subject with intact nervous system; the 2nd the patterned organized neurocontrol of flexor extensor movement; the 3rd had no organized neurocontrol.

We shall discuss how these neurocontrol patterns are generated and describe the effectiveness of different features of neurocontrol for gait.

544.14

MECHANISM OF RECOVERY FROM CEREBELLAR INCOORDINATION. H.P. Goodkin and W.T. Thach. IWJ Rehab. Institute and Depts. of Anatomy and Neurology, Washington Univ. Sch. Med., St. Louis, MO 63110.

A 61 year old man was studied during the performance of single and multijoint movements 6 weeks after right cerebellar infarct.

Wrist, instrumented single-joint: 45° ballistic flexor movements were normal except for an end point oscillation on the affected side. No statistical difference in reaction time, peak velocity, movement time or terminal position were found between performance on the two sides.

Shoulder and elbow, multi- (reach) and single-joint: the patient sat in a chair with forearms on the armrests and was instructed to reach out for a target held in front at head level. On the affected side, frame-by-frame video analysis demonstrated that reaches were performed by first elevating the arm at the shoulder and then by extending the elbow (decomposition of movement). There was no difference between single joint elbow and shoulder movements on the two sides.

Thumb and forefinger, multi- (pinch) and individuated: the patient was then asked to remove a quarter that slightly protruded from a well too narrow to admit the digits. On the normal side, the patient retrieved the coin with a precision pinch. On the affected side, the patient instead picked at the rim with the index finger in a single-digit strategy, and did not hold the other digits still.

It is commonly held that in cerebellar injury single joint angle error is the same in single and in multijoint movements (Holmes, 1939). However, we have shown in monkeys (Kane, et al. Soc Neurosci Abst. 15:52) that dentate inactivation impairs multijoint movements and spares single joint movements. Similarly, our patient used a strategy of substituting spared single joint movements for impaired multijoint movements. Thus, cerebellar damage in humans also may impair multijoint more than single joint movements. (NIH grants NS12777 and NS15070; the McDonnell Center).

544.15

STATIC ELBOW TORQUE-ANGLE RELATIONS IN HEMIPARETIC STROKE. J.P.A. Dewald, M. Munson*, T.S. Buchanan, and W.Z. Rymer. Sensory Motor Performance Program, Rehabilitation Institute of Chicago, Chicago, IL 60611.

In previous studies of muscle activation patterns in hemiparetic stroke, we observed limited changes in elbow muscle EMG activities during force exertions in three dimensions (flexion/extension, varus/valgus, and supination/pronation). In an effort to better qualitatively characterize length-tension relations in the impaired versus the unimpaired upper extremity, we evaluated torque productions for different angles of shoulder flexion/extension abduction.

Maximal forces and EMG activities were recorded at 5 angles of flexion/extension shoulder abduction ranging from -20 to 30 degrees (0 degrees = humerus was aligned with the coronal plane). The angle changes only altered the length of the biceps since no other agonists cross the shoulder joint. The elbow angle was constant at 90 degrees. The vertical shoulder abduction angle was constant at 65 degrees. The forearm was maintained in the neutral supination/pronation position. These EMG values were then used to set constant submaximal activation levels by having the subject match a given fraction of the maximal EMG at each angle. Desired levels of EMG activity along with the subject's RMS EMG were displayed on an oscilloscope. The subject was asked to maintain the RMS EMG at the desired level for 1.5 seconds.

On the unimpaired side, as angle changed from -20 to +30 degrees and the biceps shortened, elbow torque decreased 20 to 30%. There were no systematic differences in this percent reduction as a function of activation level. Similar results were obtained on the impaired side. However, torque-angle relations tended to have steeper slopes in the unimpaired versus the impaired upper extremity. The degree of this difference was variable across subjects. Less steep slopes for the impaired side would be consistent with an increased in-series compliance.

This work was supported by NIH grant NS-19331.

544.16

FINE MOTOR PERFORMANCE IN CLOSED HEAD INJURY AND DEPRESSION. Hanneke van Mier (1,4), Wouter Hulstijn (1), Everdien Tromp (2), Jac van Hoof (3) & Marion Pagen (1). 1. Nijmegen Inst. for Cogn. Res. and Info. Tech. (NICI), Univ. of Nijmegen, 2. Dept. of Rehab. Res., St. Maartenskliniek, Nijmegen, 3. Dept. of Psychiatry, Univ. of Nijmegen, The Netherlands. 4. Dept. of Neurol., Wash. Univ., Sch. of Med., St. Louis, MO 63110.

One of the characteristics shared by patients with depression (DEP) and closed head injury (CHI) is retardation of psychomotor performance. A central question is to what extent cognitive and motor factors contribute to the observed motor slowing.

An experimental method is presented that attempts to differentiate between cognitive and motor components underlying psychomotor retardation. Two figure-copying tasks, both differing in motor and cognitive complexity, were run. Task A consisted of simple line figures and task B of more complex figures. Recording of the pen movements by means of an XY-tablet (digitizer) provided objective measurements of a number of movement parameters, and made it possible to divide Movement Time (MT, time between the start and end of the drawing) in the time that the pen was on the paper (MT-down) and the time that the pen was off the paper (MT-up). MT-down was supposed to reflect motor execution and MT-up cognitive processing.

Subjects, 10 CHI patients (mean 24 yr) and 9 DEP patients (mean 50 yr), each with a matched control group, were tested on the two tasks. Both patient groups had significantly longer MT's than their controls in task B, but this was not found for the simple line drawings in task A. However, no significant difference was found between patients and controls on MT-down in task B, so the difference in MT was due to longer MT-up times for the patients. Furthermore, no interactions were found between patients and controls for motor complexity. These findings suggest that cognitive processes, rather than motor processes, are delayed. Differences in RT, errors and figure inspection are in line with these results.

545.1

FOCAL MOVEMENT VELOCITY AND PREMOVEMENT POSTURE EFFECTS ON THE ANTICIPATORY POSTURAL RESPONSE. D.L. Weeks* and S.A. Wallace. Motor Behavior Lab., Ball State Univ., Muncie, IN 47306, and Dept. of Kinesiology, Univ. of Colorado, Boulder, CO 80309.

It is unclear how the motor system self-organizes anticipatory postural muscles accompanying goal-directed upper-limb movement(focal movement) during: a)changes in focal movement velocity, or b)variations in premovement postural condition, such as locus of center of pressure, prior to focal movement initiation. The present study investigated coordination of intentional upper-limb movement concurrent with supporting postural activity in adult males during variations in arm velocity while premovement postural condition was monitored. Seven subjects performed a rapid 60 deg elbow flexion (focal movement) to a target in movement times of 170, 195, or 220 ms while standing on a force platform. Each subject adopted individual premovement postural preferences such that locus of center of pressure resided in one predominant quadrant of the foot prior to movement. Each premovement locus of center of pressure preference was accompanied by one most common postural muscle onset sequence as indicated by EMG analysis of rectus femoris and biceps femoris. In addition, onset times for postural muscles occurred earlier relative to biceps brachii onset as focal movement velocity increased. The finding that each premovement postural condition was accompanied by a particular postural muscle onset sequence was interpreted as an attempt by the motor system to constrain the degrees of freedom present in a multi-joint task by forming postural synergies. The emergent coordination may be dependent on principles of self-organization based on the concept of pattern stability.

545.2

ANTICIPATORY POSTURAL ADJUSTMENTS IN SEATED SUBJECTS. S.Moore and D.Brunt*. School of Physical Therapy, Texas Woman's University, Houston,Tx 77030.

Effects of reach velocity and postural support on anticipatory postural adjustments (APA) were analyzed in the interest of determining spatial and temporal characteristics of APA in seated persons. Increased durations of APA (EMG, kinetic and kinematic evidence) in standing subjects have been associated with fast, unsupported, loaded and unilateral arm movements. Postural muscle activity in slow and supported reaches has been reported as minimal.

15 healthy subjects (aged 24-43 years; right-hand dominant) made seated right upper extremity reaches to a target placed just within arm's length in the parasaggital plane with the right shoulder. Evidence for APA (surface EMG, kinematics, kinetics) was evaluated for two series of reaches in which maximum velocity (range 0.84-3.38 meters/sec) and 4 conditions of postural support (supported-chest belt and chair back; supported- chair back only; unsupported-erect trunk; unsupported-slumped trunk) were manipulated.

Anticipatory contralateral thoracic paraspinal (TPS) activity was present in greater than 90% of trials regardless of reach velocity or postural support. TPS preceded prime mover activity(deltoid) in 50% of all trials and preceded or occurred simultaneously with deltoid in 90% of trials. TPS onset was correlated with onsets of anticipatory vertical and A-P forces ($r=.77$, $r=.80$) exerted through the subject's chair to a force platform. Kinematic analysis revealed occasional (30%) head, trunk or leg movement prior to onset of hand movement for the fastest reaches only. These findings suggest that APA for seated subjects provide a stable upper trunk to offset the torque to be created by the mass and movement of the upper extremity. This study has implications for evaluation of postural and voluntary systems' coupling in persons unable to stand for testing.

545.3

THE RELATIONSHIP BETWEEN AGONIST AND POSTURAL ACTIVITY IN A MICROGRAVITY ENVIRONMENT. C.S. Layne, Dept. of Kinesiology, B.S. Spooner*, Bioserve Space Technologies, Kansas State University, Manhattan, Kansas 66506.

Rapid, unilateral arm raising movements are preceded by anticipatory "postural" neuromuscular activity in the back and lower limbs. Whether anticipatory postural activity is an integral component of one motor command controlling agonist activity or if agonist and anticipatory activity are independently controlled is presently not clear. A microgravity environment would presumably eliminate the functional utility of the anticipatory postural activity, thereby providing a unique environment to investigate the relationship between agonist and anticipatory activity. We used microgravity episodes during KC-135 (NASA 930) parabolic flights to collect surface EMG data from the deltoid, paraspinals and biceps femoris, during right shoulder flexions. Microgravity data was compared with baseline data collected in unit gravity. Results indicate an absence of anticipatory biceps femoris activity with anticipatory paraspinal activity remaining intact, thus suggesting biceps femoris and paraspinal activity may be components of unique postural synergies.

545.4

KINEMATIC BEHAVIOR OF HUMANS IN RESPONSE TO BASE OF SUPPORT PERTURBATIONS P.D. Hansen and M.H. Woollacott. Motor Control Lab., Uni. of Oregon, Eugene, OR 97403.

The purpose of this study was to describe the behavior of humans to postural perturbations. To do this the kinematics of 30 subjects were examined to horizontal backward perturbations (3.7 cm at 15 cm/s). Major joint movements and surface EMG from bilateral, major agonist / antagonist muscles of the leg, thigh and trunk were analyzed. Pre-perturbation subject position was controlled. Kinematic data were also collected from a model constructed of plywood and steel proportioned to a 172 cm, 73 kg male. Ankle, hip and neck joints were modeled with hinges and fixed springs.

The following conclusions were drawn from the data: 1, The initial ankle angle reversal occurred prior to EMG activity onset and was related to the termination of the perturbation; 2, There was a progressively increasing delay before the onset of joint movement in a caudal to rostral direction; 3, Two kinematic phases were discernable, a consistent phase (0 to 150-250 ms), and a subsequent variable phase; 4, The amount of movement decreased over successive trials. The mechanical model validated these findings by showing: 1, The joint movement reversed when the perturbation was terminated; 2, Increasing delays in joint movement onset in a caudal to rostral order; 3, The early phase's mechanical nature and the latter variable phase's CNS dependency.

545.5

CORRECTIVE REACTIONS TO NOVEL PERTURBATIONS OF THE HUMAN LOWER LIMB. W.E.McIlroy, J.D.Brooke and D.F.Collins, Neurophysiology Lab, University of Guelph, Guelph, Ontario, CANADA. N1G 2W1.

It is rare to find studies which report corrective reactions to completely unanticipated perturbations. The present study uses such an approach to identify contributions, to the corrective motor responses, which are strongly determined by prior planning. Subjects (n=10) were seated in a customized, leg extension apparatus. They held control loads (200 N) with instructions to keep the slide at the starting position. Control samples, taken during the static hold, acted as decoys to help ensure the first perturbation trial was completely unanticipated. A further 15 perturbation trials were later sampled at random time intervals. In all perturbation trials an external load (220 N) was added rapidly to cause whole limb flexion. EMG activity, in vastus medialis (VM) and tibialis anterior (TA), varied over the 16 perturbation trials. In contrast, activity remained constant in soleus, gastrocnemius and semitendinosus. The specificity of the influence of prior anticipation, seen in TA and VM, was observed as a significant difference in response characteristics between trials 1 and 2. Changes observed at early (<70 ms) and late latencies revealed changes in both tuning and triggered reactions. Systematic attenuation in TA and VM response magnitudes, from trials 2 to 16, revealed refinement of the prior plan (NSERC #A0025).

545.6

STEADY STATE GAINS OF POSTURAL REACTIONS TO SUPPORT SURFACE TRANSLATIONS OF STANDING HUMANS. W.A. Lee, H. Sveistrup,* M.H.Woollacott, Physical Therapy, Northwestern University, Chicago IL 60611 and Physical Education, University of Oregon, Eugene OR 97403

Postural reactions in leg and trunk muscles follow perturbations of standing humans that are induced by translations of the support surface. Postural reactions are thought to restore the person's center of mass (CM) to its initial location relative to the ankle, but the gain of this proposed feedback process has not been measured. This study will describe the steady state gains of postural reactions that follow support surface translations of different amplitudes and velocities.

Healthy young adults were perturbed by 12 sets of support surface translations (5 trials/set) that were blocked by displacement and velocity. Six anterior (A) and posterior (P) displacements were used (-6A, -4A, -2A, +2P, +4P, +6P cm) with a velocity = 4 cm/sec. Translations of +6 and -6 cm with velocities of 10, 22, and 30/sec also were applied. Set order was counterbalanced. Initial center of pressure location was controlled by feedback about AP ankle torque. A SELSPOT system recorded vertical and horizontal displacements of the ankle, knee, hip, shoulder, elbow, wrist and head, which allowed estimation of the CM. We also recorded EMG activity in the left soleus (SOL), medial gastrocnemius (GS), tibialis anterior (TA), biceps femoris (BF) and rectus femoris (RF) muscles, and AP torque. We measured the mean horizontal displacement of the CM, mean rectified EMG amplitudes, and mean AP torque for 300 ms before the translation (baseline), and for three sequential 300-ms periods after the end of the translation.

Preliminary analyses suggest that postural reactions often did not completely restore the CM to its initial location, suggesting that steady state gains were less than one, and that a new equilibrium point could be adopted after a perturbation. Steady state gains appeared to vary with the amplitude and velocity of the translation. Steady-state changes in EMG amplitudes of the SOL, GS and TA but not the BF or QD muscles were linearly related to platform amplitude or velocity ($R^2 > .94$). Further analyses and experiments will be performed to quantify CM gains and assess the reliability of the preliminary findings.

545.7

THE FREQUENCY CONTENT OF HUMAN GAIT: KINEMATIC SAGITTAL PLANE MEASUREMENTS. B. Myklebust, J. Myklebust, T. Prieto*, D. Kreis*, S. Balisreri*. Laboratory of Sensory-Motor Performance, Zablocki VA Medical Center, Medical College of Wisconsin, and Department of Biomedical Engineering, Marquette University, Milwaukee, WI 53295.

Sagittal plane (flexion-extension) movements of the hip, knee, and ankle of healthy adult subjects during level walking have been measured using a variety of data collection methods with a range of sampling rates. Knowledge of the frequency content of movements during the entire gait cycle[1] impacts on the selection of equipment for data collection, appropriate choice of sampling rates, and filtering methods to smooth individual data records.

We evaluated the frequency content of sagittal plane movements in level self-paced walking at 3 mi/hr. Data were collected simultaneously using 2 data acquisition systems: voltages from potentiometers of an electrogoniometer (Lamoreux / Orthopedic Systems, Inc.)[2] were sampled at 1000 Hz, and digitized video data (Motion Analysis Corp.) were sampled at 60 Hz. Standard observational analysis and digitized video analysis were completed simultaneously with data acquisition from the electrogoniometer in 5 gait trials of right-side limb movements. Five trials of video analysis of gait without the electrogoniometer were monitored to compare the effect of the device on gait.

While joint angle profiles for electrogoniometry and digitized video analysis are similar, differences occur in regions where movements in the transverse and frontal planes cannot be corrected in the sagittal plane. Despite significant differences in sampling rates from the video analysis and the electrogoniometer, spectral analysis of both methods demonstrate that the frequency content of gait is below 5 Hz for sagittal plane movements of the hip, knee, and ankle. Because sagittal plane movements in gait are represented by signals under 5 Hz, video sampling rates at 60 Hz are adequate. However, filtering methods for smoothing raw data must be used with care.

1. Antonsson EK, Mann RW: J Biomech 18:39-47, 1985.
2. Lamoreux LW: Bull Prosth Res BPR10-15;3-84, 1971.

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545.8

STIMULUS PARAMETERS AND PHASE DEPENDENT RESPONSES IN LOCOMOTION IN MAN. A.H. Seif-Naraghi*, J.J. Fuller*, R.M. Herman, R.A. Yapp*, S.C. D'Luzansky*, Samaritan Rehabilitation Institute, 1111 E. McDowell, Phoenix, AZ 85006; M.J. Perlow, Arizona Heart Institute, 2632 N. 20th Street, Phoenix, AZ 85006.

Phase dependent motor responses to electrocutaneous stimulation have been observed during locomotion in intact and spinal animals, and to a lesser extent in normal man (J. Yang & R. Stein, Neurosci. Abs., 14:522.3, 1988); however, human studies have not stressed the potential influence of stimulation parameters nor the reaction of the contralateral limb. This investigation was conducted to observe EMG and kinematic patterns of ipsilateral (i-) and contralateral (co-) limbs to transcutaneous posterior tibial nerve stimulation during the mid-swing and mid-stance phases of the locomotor cycle under varying stimulus conditions (pulse number, frequency, duration, intensity). M-wave of the abductor hallucis muscle was monitored to adjudge stimulus intensity. Agonist/antagonist pairs of proximal and distal muscles were evaluated. Prominent observations were (1) enhanced i-tibialis anterior (TA) and co-soleus (SOL), unchanged i-SOL and co-TA, and increased i-swing duration when stimulation was delivered during the swing phase, and (2) unchanged i-TA and i-stance duration during the stance phase. Such phase dependency was most pronounced when the stimulation conditions were 3-5 pulses, 200-300 Hz, 0.5-2.0 ms duration, and 70% of maximum M.

545.9

THE EFFECTS OF DYNAMIC VISUAL ROLL STIMULATION ON SELF-MOTION PERCEPTION AND POSTURAL CONTROL. Fred H. Previc and Thomas J. Mullen*. USAF School of Aerospace Medicine, Brooks AFB TX 78235-5301.

A widely studied visual orientation phenomenon is illusory self-motion (vection), although it may not be a good model for other visual orientation effects because it occurs at a very long latency, depends on higher-order perceptual inferences, and is cortically mediated. In this study, the latencies and magnitudes of vection and visually induced postural changes were compared using a visual roll image consisting of ~100 small white collimated squares on a wide field-of-view screen. After an initial 10-sec baseline, the image was rotated at 25 deg/sec. Subjects viewed each scene while standing on a force platform, which measured the continuous change in center-of-pressure. The latency of postural change was defined as the moment at which lateral sway first exceeded a 3-SD criterion above baseline, and was compared to vection latency. It was shown that the onset of postural change occurs within 2 sec, whereas vection commences on average around 7 sec. The two latency measures correlated significantly with each other, but not with the amplitude (magnitude) measures. These and previous findings imply that the experience of vection probably arises from both visual motion and a discounting of vestibular inputs after several seconds have elapsed. (Sponsored by Air Force Office of Scientific Research).

545.10

STABILITY OF MULTI-LOOP NEURAL CONTROL SYSTEMS. H.B. Nudelman and D.B. Rosenfield*. Stuttering Center Laboratory for Speech Motor Control, Department of Neurology, Baylor College of Medicine, Houston, Tx 77030.

A control system can become unstable i.e. "not work" even though all its components are functioning. The instability is an emergent property of the system and occurs only when the components are connected. As Norbert Wiener observed instabilities can occur in neural control systems. This means it is possible to have a neural disorder without a lesion in the classical sense.

Complex nervous systems have many interactive neural loops. Following Grimm and Nashner it is useful to distinguish between anatomic, functional, and performance loops. Functional loops perform functions that we propose must be accomplished to produce the observed behavior and performance loops are ones that are "closed" by behavior, i.e. a muscle spindle measures the length of a muscle and feedsback to the motoneuron pool of that muscle. One measures the behavior of a "performance loop" that is driven by a group of proposed "functional loops" that are composed of a subset of the possible anatomic loops. To consider instability we lump the system into a functional loop that is driving a performance loop.

The dynamics of the performance loop can be measured. We assume they can be approximated by a linear term and a nonlinear term and that the phase margin of the linear term is specified. Neurophysiology tells us there will be time delays in the outer loop. These time delays effect a phase lag into the total systems transfer function. This phase lag is equal to the time delay divided by the period of the sinusoidal component under consideration.

The system will become unstable whenever this phase lag equals the phase margin on the performance loop. Biological examples will be given.

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545.11

HFOS IN BILATERAL AND IPSILATERAL SYNERGIST MUSCLE PAIRS IN HUMAN MASTICATION. M. Denny* and A. Smith. Audiology & Speech Sciences Dept., Purdue University, West Lafayette, IN 47907.

Correlated high frequency oscillations (HFOs) observed in respiratory-related neural and EMG activity are thought to represent a widely distributed output of the respiratory CPG (e.g., Bruce & Ackerson, 1986). We have proposed that bilaterally correlated HFOs observed in human masseter muscles during mastication may represent a similar phenomenon resulting from the action of a masticatory CPG (Smith & Denny, J. Neurophysiol, 1990). If HFOs observed during chewing represent a distributed output of a CPG, they should appear in ipsilateral as well as bilateral synergist muscle pairs. If correlation of HFOs increases with neural drive, ipsilateral correlations may be higher on the working side. Activity was recorded from jaw-closing muscles during natural chewing and chewing controlled for working side. Power and coherence spectra were calculated to estimate the strength of correlation between bilateral and ipsilateral pairs. Preliminary analyses indicate that ipsilateral pairs of jaw-closing muscles show significantly correlated activity during mastication.

545.12

CONTROL OF HUMAN JAW MOVEMENT IN MASTICATION AND SPEECH. D.J. Ostry, K.G. Munhall*, J.R. Flanagan and A.G. Feldman. McGill University, Montreal, Canada and Institute for Information Transmission Problems, Moscow, USSR.

The X-ray microbeam was used to record jaw movement kinematics in the mid-saggital plane. The jaw movements were examined in terms of the rotation of the condyle and the translation of its axis of rotation along the articular eminence. Mastication trials employed rubber tubing in which compliance and diameter was systematically varied. In speech trials, consonant-vowel combinations were produced at different rates and loudnesses. It was found that when movements of the jaw in mastication were plotted in joint coordinates, the relationship between jaw rotation and jaw translation was essentially fixed. However, when jaw movements in speech were examined, the relationship between rotation and translation was not constant but varied in a systematic way with the composition of the utterance. The evidence from speech suggests that the nervous system is capable of altering the relationship between jaw rotation and jaw translation. Microbeam recordings were also used to recover bite force during the cycle by relating the recorded separation of the teeth at the point of contact with the bolus to separately measured tension-compression functions for each of the tubes. Simulations of human jaw movement based on the equilibrium point hypothesis were found to adequately capture both free and compliant motion phases of orofacial movements. The model allows for changes in coordination between translation and rotation as well as the control of stiffness.

545.13

HUMAN MOTOR UNIT FIRING DURING QUASI-SINUSOIDAL ISOMETRIC MUSCLE CONTRACTIONS. M.B. Iyer, C.N. Christakos and C. Ghez. Center for Neurobiology and Behavior, Columbia University and New York State Psychiatric Institute, NY 10032.

This study was designed to investigate the role of motor units (MUs) in the generation of time-varying isometric muscle forces in humans. Subjects produced nearly sinusoidal contractions of the first dorsal interosseus muscle of the hand about fixed force levels (range 15% to 55% of maximal voluntary contraction - MVC), with frequencies in the range 0.25 Hz to 5 Hz and amplitudes in the range 6% to 30% MVC. Single-MU discharges and multi-unit EMG were recorded from the muscle and subjected, together with the force signal, to spectral analysis. The auto-spectra of single-MU activities, as well as of multi-unit EMG, showed a modulation of the discharge rates of the units, with the carrier rates (MU mean discharge rates) remaining more or less constant for a given level of contraction. This rate modulation of MU firing was at the frequency of the sinusoidal variation of the muscle force and showed a phase advance over it. The modulating component in a unit's auto-spectrum increased in size as the depth of the modulation of the force increased. Further, such modulating components were much stronger for multi-unit EMG than for single-MU discharges, which implies correlations between the modulations of different MUs. This was verified by computing coherences between the various signals, which showed large values at the common modulation frequency. Indeed, high coherences between single units, subsets of units, and the entire population signify correlations between unitary activities. These observations therefore strongly suggest that a time-varying isometric muscle force is produced by correlated variations of MU discharge rates, caused by a common, time-varying drive to the alpha-motoneuron pool. (Supported by NS 22715)

545.14

ESTIMATING THE STRENGTH OF COMMON INPUTS TO MOTOR NEURONS FROM THE CROSS-CORRELOGRAM. M.A. Nordstrom[1], A.J. Fuglevand[2], R.M. Enoka[1,2] and K.S.Türker*[3]. Dept. Physiology[1] and Dept. Exercise & Sport Sciences[2], Univ. of Arizona, Tucson AZ 85724 and Dept. Physiology[3], Univ. of Adelaide, Adelaide 5001, Australia.

Common synaptic input to motor neurons produces a degree of synchronization of discharge times which is related to the strength of the common input. A method for deriving the strength of common synaptic input (CIS) to pairs of human motor neurons (in terms of average amplitude and input frequency of common EPSPs) has been developed from measures of synchrony in the cross-correlogram using a model of the membrane trajectory between spikes (Miles, T.S. et.al., *Exp. Brain Res.* 77:628,1989). The model can also explain the dependence of the size of the synchronization peak in the cross-correlogram on the motor neuron discharge rates. In the present study, the predictions of the model were compared to empirical relationships obtained from cross-correlation of pairs of human first dorsal interosseous motor units that were voluntarily activated at different mean rates (range 7.5 - 18 Hz). Unlike other measures of correlation derived from the cross-correlogram, the CIS is not directly influenced by the respective motor neuron firing rates, and also describes the correlated activity in terms of the underlying physiological processes (i.e. frequency and amplitude of common EPSPs). Due to this independence, the CIS is the preferred descriptor of correlated activity of motor neurons under conditions in which the relative discharge rates of the motor neurons change with time, such as with a fatiguing voluntary contraction. Supported by USPHS grants NS 07309 and NS 20544. M.A.N is a C.J. Martin Fellow of the NH&MRC of Australia.

545.15

THE FATIGABILITY OF SINGLE MOTOR UNITS IS DEPENDENT IN PART ON THE PATTERN OF ACTIVATION. Y. Laouris, L. Bevan, R.M. Reinking and D.G. Stuart. Department of Physiology, College of Medicine, University of Arizona, Tucson, AZ, 85724.

To assess motor unit (MU) fatigue, trains of constant-frequency stimuli have conventionally been delivered to motoneurons or their axons, either repetitively or continuously (e.g.: J. Physiol. (Lond). 234:723, 1973; Brain Res. 327:203, 1985). However, observations of MU behavior in conscious humans suggest that their frequency of activation is not constant, even when muscle force is constant (J. Physiol. (Lond). 340:335, 1983; J. Exp. Biol. 115: 125, 1985). Recently, we reported that MU force can be augmented during fatigue by subtle changes in the activation pattern and that the efficacy of such changes increases as fatigue ensues (Soc. Neurosci. Abstr. 15:396, 1989). We now report an extension of this finding by assessing four indices of fatigue for the two activation patterns. The patterns were 500 ms in duration and delivered in random alternation at 1/s to the ventral-root axons of 22 fast-fatigable (FF) MUs of the tibialis posterior muscle from 6 cats during a 4-min fatigue test. One pattern (*regular*) was comprised of a constant-frequency train (interstimulus intervals set at 1.8 x contraction time of the unit; usually 15-30 Hz). The other (*optimized*) pattern, contained the same number of stimuli. However, the initial part of this train contained a triplet (three stimuli separated by 10 ms intervals). The overall force records were decomposed to extract the force profiles attributable to each pattern (Soc. Neurosci. Abstr. 15:396, 1989). The indices of fatigue used to quantify fatigability were: 1) a progressive fatigue index; 2) a cumulative force index; 3) the time to 50% of the initial force; and 4) the time to 50% of the total cumulative force-time integral. The results indicate that MUs are relatively less fatigable when stimulated with an optimized pattern rather than a regular pattern, irrespective of the way in which fatigability is quantified. Supported by USPHS grants NS 07309, HL 07249, NS 25077, and RR 05675.

545.16

USE OF FLUTTER FREQUENCY PERIODIC TORQUE MODULATION AS A MONITOR OF Ia REFLEX GAIN DURING MOVEMENT J.S. Thomas, Department of Physiology, Meharry Medical College, Nashville, TN 37208

Flutter frequency (10-50 Hz) periodic torque modulation can be used as a "sounding signal" to monitor Ia reflex gain during ongoing movement. Averaging of averaged sets of EMG and movement parameter records, obtained with torque modulations of opposite phase relative to the TASK imperative signal (target jump and/or discrete torque change), allows unambiguous recovery of the EMG activity and movement parameters associated with the TASK movement. Comparison of these "demodulated" EMG envelopes with those from the same TASK, but without periodic torque modulation, indicates that superimposed flutter frequency perturbation had NO EFFECT on TASK related performance. Subtraction of "phase180" from "phase0" data sets removes TASK specific responses and allows cycle by cycle assessment of the amplitude of EMG and movement parameter response to periodic perturbation during the TASK movement. Typically the "volitional" phases of TASK movement show a profound inhibition of periodic EMG modulation (assumed to reflect Ia reflex gain), sometimes followed by an enhancement of periodic reflex gain as the new posture is stabilized.

545.17

MOTOR UNIT ACTIVATION IS NOT TOPOGRAPHICALLY LOCALIZED IN HUMAN MUSCLE. G. Kamen, Y. Masakado*, C. J. De Luca. NeuroMuscular Research Center, Boston University, Boston, MA 01701.

In an effort to investigate the notion that muscles may be organized into functionally distinct neuromuscular compartments, the independence of motor unit action was assessed in different regions of the human tibialis anterior (TA) muscle. Motor unit recordings were obtained from the TA of five subjects using two quadrifilar needle electrodes inserted 5-6 cm apart. During isometric contractions at 30% MVC, signals obtained from each needle were decomposed to obtain individual motor unit action potential firing trains. Motor unit firing histories from within each needle site and between the two sites were studied to determine the frequency of simultaneous (synchronous) discharges, and to determine the level of common fluctuation of firing rates. These analyses indicated a high degree of synchronization at zero or near-zero latency from units recorded in both distal and proximal needle sites and a similar level of synchronous firing among units measured between the two sites. Also, the fluctuation of firing rates was similar among distal, proximal and between-needle motor units, with cross-correlations of firing rate trains of about 0.6 obtained in each case. These data suggest the existence of a strong central command to all motor units within a given muscle, without regard to motor unit topographical organization.

This work was sponsored by a grant from the Rehabilitation Research and Development Service of the Veterans Administration.

546.1

Sexually Dimorphic Spatial Abilities in the Morris Water Task. S.G. Warren , L.A. Wilson and L. Nadel. Dept. of Psychology, Univ. of Arizona, Tucson, AZ 85721.

One consistently observed difference between the sexes concerns spatial ability. Males have generally been observed to outperform females on tasks that require them to acquire and retain spatial information. Several possible explanations for these differences have been suggested. First, males might make more efficient use of the euclidian geometric properties of the environment while females rely more heavily on information provided by single landmarks or cues. Second, males could prefentially use distal landmarks or cues while females' spatial behavior is controlled primarily by proximal landmarks or cues. This notion is consistent with the ecological view that the ability of male rats to utilize distal cues more efficiently reflects the larger territory through which they roam, whereas females spend their reproductive life in a more restricted environment. The present study hypothesized that males would perform better than females on a spatial task requiring the use of distal cues whereas females would perform at least as well as males when only proximal cues were available. Forty-nine male and female Long-Evans hooded rats were tested on two versions of a water-maze task. In the proximal condition a curtain was hung just outside the perimeter of the tank. The curtain was marked with eight cues. The curtain was removed for the second condition, thus forcing subjects to rely on distal information within the larger environment. This information included cues placed around the room. There were no differences between males and females when the curtain was in place, and only proximal cues available. Males performed significantly better when the curtain was removed, and distal cues available. This advantage was not evident for the females. These results suggest that males have an advantage when the situation permits the use of distal cues; however when only proximal information is available males and females do not differ in their performance on a spatial task.

546.2

A NEURAL NETWORK APPROACH TO HIPPOCAMPAL FUNCTION IN CLASSICAL CONDITIONING. Nestor A. Schmajuk and James J. DiCarlo*. Department of Psychology, Northwestern University, Evanston, IL 60208.

We describe hippocampal participation in classical conditioning in terms of Grossberg's (1975) attentional theory. According to this theory, pairing of a conditioned stimulus (CS) with an unconditioned stimulus (US) causes both an association of the sensory representation of the CS with the US (conditioned reinforcement learning) and an association of the sensory representation of CS with the drive representation of the US (incentive motivation learning). Sensory representations compete among themselves for a limited-capacity short-term memory (STM) that is reflected in a long-term memory (LTM) storage.

We introduce the "STM regulation" hypothesis which proposes that the hippocampus controls incentive motivation, self-excitation, and competition among sensory representations thereby regulating the contents of a limited capacity STM. Under the "STM regulation" hypothesis, we map nodes and connections in Grossberg's neural network onto a three-dimensional hippocampal circuitry. The resulting neural model provides (a) a framework for understanding the dynamics of information processing and storage in the hippocampus and cerebellum during classical conditioning of the rabbit's nictitating membrane, (b) principles for understanding the effect of different hippocampal manipulations on classical conditioning, and (c) numerous novel and testable predictions.

546.3

IMPROVED PERFORMANCE ON DRL TASKS IN RATS WITH HIPPOCAMPAL LESIONS TREATED WITH THE CALCIUM ENTRY BLOCKER, NIMODIPINE. S. Finger, L. Green*, M. Tarnoff*, K. Mortman* and A. Anderson*. Department. of Psychology, Washington University, St. Louis, MO 63130.

Rats were trained to lever press and then were given either bilateral electrolytic lesions of the hippocampus or control operations. Half of the rats in each group received oral nimodipine, while the remaining animals received vehicle, for the 14 days following surgery. The rats were then tested on a DRL-20 sec schedule of reinforcement that required them to withhold a lever press for 20 sec in order to earn a liquid reward. Rats with lesions not given nimodipine performed very poorly while those treated with the drug performed well within the control group range. Similar trends were obtained when the rats were advanced to a DRL-40 sec schedule one month later. These findings show that nimodipine can attenuate the behavioral effects of large hippocampal lesions on even difficult memory tasks.

546.4

DIFFERENTIATION OF PASSIVE AVOIDANCE DEFICITS PRODUCED BY LESIONS OF THE CENTRAL AMYGDALOID NUCLEUS OF THE RAT. G.D. Coover and B.W. Santi*. Department of Psychology, Northern Illinois University, DeKalb, IL 60115.

Electrolytic but not ibotenate lesions placed rostrally in the central amygdaloid nucleus (rostral ACE) produce a very marked deficit in passive avoidance (PA) of drinking (Coover et al., Soc. Neurosci. Abstr., 15:1251, 1989). The present study with electrolytic lesions addresses the possible contribution of more central neurons of the ACE nucleus, and uses an additional PA task.

Rats with rostral ACE lesions required 33.1 ± 2.9 (Mean ± SEM) footshocks of an ascending series of intensities to passively avoid drinking for 5 min. In contrast, control rats required only 18.7 ± 1.4 footshocks ($p < .001$). Rats with lesions aimed at the middle of the ACE nucleus (middle ACE), just 1.0 mm caudo-ventral to rostral ACE coordinates, required only 22.7 ± 1.9 footshocks, fewer than rostral ACE ($p < .05$) but not more than controls.

In contrast, both lesion groups exhibited deficits in a one-trial step-through PA task. Of the controls, 10 of 11 remained in the brightly lit chamber for the maximum of 10 min on the test trial one day after receiving a footshock for stepping into the dark chamber, while 0 of 8 rostral ACE and 1 of 6 middle ACE rats did so ($p's < .02$).

The amygdala plays some role in PA behavior, but electrolytic lesions of some portions may cause a more profound deficit for which ACE neurons aren't responsible.

546.5

SELECTIVE AND TRANSIENT BEHAVIORAL BENEFITS ARE PRODUCED BY NEURAL GRAFTS THAT PROMPTLY FOLLOW RADIATION-INDUCED HYPOPLASIA OF FASCIA DENTATA GRANULE CELLS. G.A. Mickley[1,2], J.L. Ferguson[1], T.J. Nemeth*[1,2] and B.A. Barrett[1]* BHS, AFRRI, Bethesda, MD 20814 USA[1] and USAFSAM, Brooks AFB TX 78235[2].

X-irradiation of the neonatal rat hippocampus produces a selective hypoplasia of fascia dentata granule cells, locomotor hyperactivity, perseverative movements and deficits in passive avoidance. Transplantation of fetal hippocampal neurons into the adult (age=182±4 days) brain produced a partial behavioral recovery. (Mickley et al., Brain Res., 509:280, 1990).

Since graft/host interconnections are more prominent when transplants are conducted soon after brain damage, we transplanted hippocampal or cerebral cortex neurons when host rats were 33±5 days of age (i.e., only 16 days after radiogenic brain damage). Behavioral evaluations were conducted 80 and 182 days after grafting or surgical control procedures.

Hippocampal transplants failed to reduce deficits in passive avoidance. However, in the first test series only, selective components of locomotion (e.g., stereotypy) and perseverative turning (e.g., mean bout length and rapid turning) were improved by the grafts. These data suggest that the timing of neural graft placement may influence the pattern and duration of behavioral recovery.

547.1

ICI D7569: A CINNOLINE CARBOXAMIDE ANXIOLYTIC WITH PARTIAL AGONIST ACTIVITY AT THE BENZODIAZEPINE RECEPTOR. J.B. Patel, A.I. Salama, J.B. Malick, J.F. Resch*, D.C. U'Prichard, R.E. Giles, B. Hesp*, B.M. Meiners, and M.E. Goldberg. ICI Americas Inc., Wilmington, DE 19897.

ICI D7569 (ID) exhibited potent anticonflict activity in the rat (MED = 0.8 mg/kg) as well as in monkeys (MED = 6.25 mg/kg) procedures predictive of anxiolytic activity in man. The compound showed no effects on rotorod performance in rat up to 200.0 mg/kg, p.o. indicating non-sedative profile. It is less likely to potentiate the sedative actions of ethanol than benzodiazepines (BZ). In addition, ID demonstrated potent oral activity (MED = 1.0 mg/kg) against metrazole-induced convulsions in rats. Of particular interest, and unlike diazepam, chronic ID treatment did not cause an antagonist (RO 15-1788) precipitated withdrawal syndrome (seizures) in mice, suggesting lack of physical dependence liability. Furthermore, ID (12.5 mg/kg, p.o.) antagonized diazepam-induced sedation suggesting partial agonist activity.

Neurochemically, ID is potent (IC_{50} = 0.31 nM) and selective for Type 1 (cerebellar) BZ receptor. Since, GABA increases the affinity of ID at the BZ binding site and its potency is reduced by photoaffinity labeling, this suggests that it has agonist properties at the BZ receptor. Thus, ID should be a potent non-sedative partial agonist anxiolytic in man with considerably reduced physical dependence liability compared to BZ.

547.2

U-78875, A BENZODIAZEPINE ANTAGONIST WITH ANXIOLYTIC PROPERTIES IN RATS AND MICE. P.J.K.D.Schreur, N.F.Nichols, J.F.Pregenzer*, and J.A.Oostveen*. CNS Research, The Upjohn Company, Kalamazoo, MI 49001.

U-78875 {3-(5-cyclopropyl-1,2,4-oxadiazol-3-yl)-5-(1-methyl-ethyl)-imidazo-(1,5-a)quinoxalin-4(5H)-one} is a non-benzodiazepine which binds with the benzodiazepine (BZ) receptor and has some antagonist and agonist properties. We compared U-78875, Ro 15-1788, diazepam, and alprazolam in 5 anxiolytic tests.

In Upjohn-reared Sprague-Dawley rats, U-78875 is active in the center test (increases time away from the walls of a cage) and hole-board (HB) test (increases number of holepokes) and causes locomotor disinhibition (LD) (increases exploratory locomotor activity) at doses of 1-10 mg/kg s.c. In CF-1 mice, U-78875 increases the amount of social interaction in the face to face test (10 mg/kg orally) and suppresses isolation-induced aggression (10 mg/kg i.p.). However, in C57BL/6 mice, it does not impair rotarod performance even at 100 mg/kg i.p. Flumazenil (Ro 15-1788), the classic BZ antagonist, is inactive as an anxiolytic in the center, HB, LD, and face tests (it was not tested against aggression). Diazepam (Valium) was active in all 5 anxiolytic tests (1-10 mg/kg in 4 tests, 0.1 mg/kg or more against aggression). Alprazolam (Xanax) was 3-30 times more potent than diazepam in 4 anxiolytic tests (not tested against aggression). Diazepam and alprazolam impaired rotarod performance.

In conclusion, U-78875 is anxiolytic in a variety of rodent models, but does not have the sedative activity of classical BZ agonists.

547.3

EFFECTS OF DIAZEPAM ON THE STRESS-INDUCED INCREASE IN EXTRACELLULAR DOPAMINE AND NOREPINEPHRINE IN MEDIAL PREFRONTAL CORTEX. Janet M. Finlay, Michael J. Zigmond and Elizabeth D. Abercrombie. *Dept. of Behavioral Neuroscience, University of Pittsburgh, Pittsburgh, PA, 15260.*

A variety of stressors increase extracellular dopamine (DA) and norepinephrine (NE) in medial prefrontal cortex (mPFC) and these neurochemical responses may play a role in the etiology of clinical anxiety. We therefore assessed the effects of the anxiolytic benzodiazepine diazepam on the stress-induced release of catecholamines in mPFC. Simultaneous determination of extracellular DA and NE concentrations was performed using *in vivo* microdialysis in freely moving rats. Rats were given an injection of either diazepam (2.5 mg/kg, IP) or its vehicle and 1 h later subjected to 30 min of tail-pressure stress. Diazepam alone elicited a 60-70% decrease in basal extracellular DA and NE concentrations. When this diazepam-induced shift in basal DA and NE concentrations was accounted for, tail-pressure stress elicited an 80-90% increase in DA and NE levels in both the vehicle and diazepam pre-treated rats. Thus, whereas diazepam does not eliminate the response to stress, it may exert its anxiolytic effects by decreasing the absolute magnitude of DA and/or NE release in mPFC during stress. Additional experiments will examine the effects of diazepam on the stress-induced increase in NE and/or DA in the hippocampus and amygdala, sites which also have been implicated in anxiety and in the mechanism of action of anxiolytics. *(Supported by an MRC of Canada Postdoctoral Fellowship to JMF, USPHS Grant MH45156 and MH43947, and a gift of diazepam from Hoffman-La Roche.)*

547.4

THE EFFECTS OF ORAL IDAZOXAN VS YOHIMBINE IN HEALTHY SUBJECTS. C.J. McDougle, M.D., J.H. Krystal, M.D., S.W. Woods, M.D., L.H. Price, M.D., D.A. Herbst, B.S., G.R. Heninger, M.D., D.S. Charney, M.D., Yale Univ. Dept. of Psychiatry, 34 Park St., New Haven, CT 06519.

The primary purpose of this study was to investigate the effects of oral Idazoxan, an alpha-2 adrenoceptor antagonist, on behavior and norepinephrine (NE) turnover in healthy subjects. In addition, the study sought to determine if the biochemical and behavioral effects of Idazoxan are similar to those of the alpha-2 adrenoceptor antagonist yohimbine. Methods: Ten healthy male subjects received randomized, double-blind oral administration of placebo, 20 mg, 40 mg, and 80 mg of Idazoxan as well as yohimbine 20 mg, on five separate test days. Blood samples for plasma 3-methoxy-4-hydroxyphenylglycol (MHPG) (ng/mL) and cortisol (ug/dL), vital signs, and behavioral ratings were obtained at baseline and at intervals for up to 4 hrs following the oral study dose. Results: The 20 mg (1.31±0.84, p<.001), 40 mg (1.21±0.95, p<.003), and 80 mg (2.04±1.34, p<.002) doses of Idazoxan resulted in consistent, significant increases in plasma free MHPG. The 20 mg dose of yohimbine also resulted in a significant increase in plasma free MHPG (1.76±1.12, p<.002). Plasma cortisol levels were significantly increased following the 80 mg dose of Idazoxan (5.34±7.35, p<.05) and following the 20 mg dose of yohimbine (4.61±6.54, p<.05). Systolic and diastolic blood pressure (sitting and standing) increased significantly following all three idazoxan doses and following yohimbine. Conclusion: The robust increase in plasma MHPG following all three doses of oral Idazoxan demonstrates that Idazoxan increases NE turnover in human subjects. Although Idazoxan and yohimbine may differ in their selectivity for alpha-2 receptor subtypes, this study suggests that neuroendocrine and behavioral responses to these drugs are similar in human subjects. The measurement of the effect of Idazoxan on NE turnover and behavior may provide a means of assessing alpha-2 adrenoceptor function in human subjects.

547.5

BEHAVIORAL EFFECTS OF TANDOSPIRONE IN MALE BABOONS. C.A. Sannerud[1], N.A. Ator[1], C.T. Fischette[2] & R.R. Griffiths[1]. Johns Hopkins Univ. Med. Sch.[1] & Pfizer Pharmaceuticals[2].

Tandospirone [T (SM3997) Pfizer Pharmaceutics Inc.] is a pyrimidinylpiperazine compound with activity at 5-HT_{1A} binding site which is under development as an anxiolytic/antidepressant. Four baboons were maintained on 50 mg/kg/day T via continuous i.g. infusion for 7 consecutive weeks. Chronic T produced few behavioral signs of sedation; lip droop was seen in only 2 baboons. There was equivocal evidence that chronic T changed the sensitivity to flumazenil. Flumazenil (5 mg/kg, i.m.) given on day 8 of chronic T increased limb tremor in 2 baboons and body tremor in 1 baboon, but did not produce other signs typical of benzodiazepine (BZ) precipitated withdrawal (WD). Vehicle substitution after week 7 produced transitory increases in signs of limb tremor and abnormal postures, and decreases in food intake. No signs of vomit or myoclonic jerks/seizures were observed in any baboon. These patterns of behavioral change indicated a very mild BZ-like WD syndrome that was less severe than from lorazepam (LOR) or diazepam. The discriminative stimulus effects of T were assessed in baboons trained to discriminate 1.8 mg/kg LOR, (n=3) or 10 mg/kg pentobarbital (PB, n=3) from no drug. BZ compounds generally show dose-related increases in drug-lever responding in LOR or PB trained baboons. T (0.1-32 mg/kg), however, did not occasion drug-lever responding in either training group after i.m. or p.o. administration. T was more potent at decreasing response rates when given i.m. than p.o. Taken together, T appears to have behavioral effects in baboons different from BZ agonists.

547.6

AUTORADIOGRAPHIC DISTRIBUTION OF 5-HT1A RECEPTOR BINDING SITES FOLLOWING SUBCHRONIC TREATMENT WITH IPSAPIRONE. K. McMonagle-Strucko* and R.J. Fanelli. Institute for Preclinical Pharmacology, Miles Inc., West Haven, CT 06516.

Ipsapirone has been shown to have potent anxiolytic and antidepressant properties in a variety of animal models. Ipsapirone, a high affinity ligand for the 5-HT1A receptor subtype, is a full agonist at presynaptic serotonergic sites and a partial agonist at postsynaptic sites. Experiments were done to determine whether treatment with ipsapirone would differentially affect binding to 5-HT1A receptors at these different sites. Rats were treated twice daily with ipsapirone (10 mg/kg ip) for 14 days. Quantitative analyses were done of autoradiograms of in vitro [3H]8-OH-DPAT binding (1 nM, with or without 10 μM 5-HT, apposed to [3H]-sensitive film for 5 weeks) to selected brain regions. Binding in untreated rats was highest in the hippocampus, septum, entorhinal cortex and raphe nuclei. Subchronic ipsapirone treatment resulted in a large decline ($\cong$ 50%) in binding in raphe nuclei and to a lesser extent in the entorhinal cortex, without altering other regions analyzed. These data support the hypothesis that region specific effects of ipsapirone contribute to its behavioral profile.

547.7

THE 5-HT1A AGONIST, 8-OH-DPAT, STIMULATES FOOD INTAKE OF RHESUS MONKEYS. S.M. Pomerantz, Dept. of Physiology, Univ. of Pittsburgh School of Medicine, Pittsburgh, PA 15261.

There is a great deal of interest in evaluating pharmacological agents which may be clinically applied in the treatment of eating disorders. In the present study, we developed a simple feeding paradigm to be used in primate species to examine the effects of pharmacological manipulations on food intake. Adult male rhesus monkeys were placed on a feeding schedule in which a main meal of Purina monkey chow was provided at 1000 hr and a supplemental meal of monkey chow was provided another time during the day. Monkeys generally ate most of their food during the first two hours following their main meal, but also "snacked" following supplemental food presentation. This supplemental feeding period was used to evaluate the effects of the 5-HT1a agonist, 8-OH-DPAT (DPAT). DPAT (10 μg/kg) increased food intake when administered at either 2 hr or 6 hr after the main meal, but not when administered immediately prior to the main meal. Facilitation of supplemental food intake by DPAT was most prominent during the first hour after its administration, but by 4 hours after its administration, food intake returned to control levels. Dose-response analysis revealed that DPAT exhibited a potent biphasic effect during the first hour after its administration (n=7), with low doses (5, 10, and 25 μg/kg) reliably stimulating food intake 186, 208, and 212%, respectively over control vehicle levels, and a high dose of DPAT (200 μg/kg) inhibiting food intake to 51% of control levels. These results indicate that pharmacological agents that interact with 5-HT1a receptors influence food intake in a primate species and that such agents may be clinically efficacious in humans in controlling food intake.

547.8

DOWN REGULATION OF BRAIN 5-HT_2 RECEPTORS UNDERLIES ANXIOLYTIC EFFECT PRODUCED BY SUSTAINED TREATMENT WITH GEPIRONE. D. Benjamin[1,3], E.I. Saiff[3], H. Lal[1], and J. Coupet[2]. [1]Texas Coll. of Osteopathic Med., Ft. Worth, TX and [2]Am. Cyanamid Med. Res. Div., [3]Ramapo Coll. of New Jersey, Mahwah, NJ.

Acute administration of gepirone, a 5-HT_{1A} agonist, produces anxiolytic-like effects in mice and rats. Gepirone also produces a treatment duration-dependent anxiolytic-like effect that persists for up to four days following its elimination. Our study was designed to characterize the alterations in serotonergic receptors which are produced by sustained treatment with gepirone, and which are related to the onset and continuation of the anxiolytic-like effect. Mice were injected chronically with gepirone, 15 mg/kg, bid, and after discontinuation of the treatment, performance on behavioral tests, and *in situ* receptor binding were assessed. In the gepirone-treated mice, a significant anxiolytic-like effect as revealed by specific performance in the elevated plus-maze was observed at 24-96 h following 7, 14 or 21, but not 1 d of treatment. There was a significant decrease (up to 50%) in the density of cortical 5-HT_2 receptors. No change was detected in 5-HT_{1B} receptors or in amine release sites; 5-HT_{1A} binding increased, but this effect was preceeded by the behavioral changes. Head-shakes induced in mice by 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI; 1mg/kg), thought to reflect 5-HT_2 receptor function, were virtually abolished following 7, 14 and 21 d of gepirone treatment, but not after 1 d of treatment. 5-HT_2 receptor down regulation and decrements in the head-shake response to DOI were correlated with the anxiolytic-like effect, thus suggesting that decrements in the density and function of 5-HT_2 receptors underlie the anxiolytic-like effects of gepirone. Supported partially by NIAAA grant RO1 3521.

547.9

INTERACTION OF ADRENOCEPTOR ANTAGONISTS WITH 5-HT_{1A} AGONISTS IN DRUG DISCRIMINATION. L. Zhang and J. E. Barrett. Dept. of Psychiatry, Uniformed Services Univ. of the Health Science Bethesda, Maryland 20814

In order to investigate the mechanism of action of novel anxiolytic drugs such as buspirone, the beta- and alpha-adrenoceptor blocking drugs, pindolol and prazosin were studied in a drug discrimination procedure. Pigeons were trained to discriminate 8-OH-DPAT (0.3 mg/kg) from saline. Dose-response curves were tested with the drugs before and after chronic administration of 8-OH-DPAT (3.0 mg/kg/day for 4 to 6 weeks). Dose-response curves for 8-OH-DPAT and buspirone were shifted to the left after chronic administration. Prazosin did not generalize before or after chronic injection. Pindolol did not produce drug key responding before but it did so after chronic administration of 8-OH-DPAT. Although some report that pindolol has 5-HT receptor antagonist effects, it may have partial 5-HT_{1A} receptor agonist-like properties. After 8-OH-DPAT chronic administration, the 5-HT_{1A} receptor may be more sensitive to pindolol. The results indicate that alpha-1 antagonist properties are not directly involved in mediating discriminative stimulus effects of 8-OH-DPAT. 8-OH-DPAT and the beta-adrenoceptor blocking agent pindolol can share discriminative properties under certain conditions. Our approach may provide a useful behavioral model to investigate the mechanism of anxiolytic effects of 5-HT_{1A} agonist. (Supported by DA-02873).

547.10

REGION-SPECIFIC TOLERANCE TO THE 5-HT_{1A} AGONIST IPSAPIRONE. M.F.Piercey, Y.Tian*, J.T.Lum, W.E.Hoffmann, R.J.Collins*, M.M.Cooper*, K.Lookingland, and K.E.Moore. The Upjohn Company, Kalamazoo, MI USA and Michigan State University, E. Lansing, MI USA.

In order to better understand delayed therapeutic onsets of 5-HT_{1A} anxiolytics, we evaluated acute ipsapirone (IPS) in animals treated with chronic IPS (15 mg/kg/day i.v. infusion, 7 days). In chronic saline animals, IPS depressed 5-HT neuron firing (dorsal raphe, ED_{50} = 12 μg/kg i.v.), 5-HIAA/5-HT ratios, body temperature and regional brain energy metabolism (2-DG autoradiography), especially in hippocampus, cortex, basal forebrain and raphe areas. In chronic IPS animals, acute drug was less potent in depressing 5-HT neuron firing (ED_{50} = 53 μg/kg) and slightly less effective in producing hypothermia. Tolerance was observed to IPS's depression of 5-HIAA/5-HT ratios in spinal cord, but results were inconclusive elsewhere. In 2-DG autoradiography, acute effects were depressed by chronic treatment in some regions (e.g. prefrontal cortex, m. hypothalamus, basal forebrain, etc.) without being significantly affected in others, including the hippocampus. It is concluded that tolerance differentially develops among brain regions and this could be relevant to the delays observed in anxiolytic activities of the drug.

547.11

EFFECTS OF β-ADRENOCEPTOR ANTAGONISTS ON SEROTONIN METABOLISM AND AGGRESSION IN ISOLATED MICE. J.K. Chamberlain and J.P. DaVanzo. Department of Pharmacology, School of Medicine, East Carolina University, Greenville, NC 27858

β-adrenoceptor antagonists are known to inhibit isolation-induced fighting behavior in mice. Recent work correlated this effect with affinity for serotonin (5-HT)1a receptors and not β-receptors. Activation of 5-HT_{1a} receptors is known to decrease serotonergic impulse flow and the β-adrenoceptor antagonist propranolol is known to antagonize this effect. The effect of acute and chronic β-antagonist administration on 5-HT metabolism was examined in isolated fighting mice. Acutely, d,l-penbutolol significantly increased 5-HT levels in olfactory bulbs and hippocampus. Administration for ten days had no effect on 5-HT levels, but 5-hydroxy indole acetic acid (5-HIAA) was increased in septum and hippocampus of mice treated with d,l-penbutolol. Propranolol, l-penbutolol, and d-penbutolol at behaviorally effective doses had no effect on 5-HT or 5-HIAA levels in any group tested. Since decreased serotonergic activity is associated with decreased aggression, increased serotonin metabolism as indicated by increased 5-HIAA levels is probably not the mechanism by which β-antagonists inhibit fighting behavior. (Supported by Hoechst-Roussel Pharmaceuticals Inc.)

547.12

SIGNIFICANT SEDATIVE EFFECTS REPORTED WITH TWO PUTATIVE NONSEDATIVE ANXIOLYTIC DRUGS USING THE ACCELERATING ROTO-ROD. R. C. Meyer, C. E. Lints and W. J. Kasprow*. Department of Psychology, Northern Illinois University, DeKalb, IL 60115.

Previous research with the drugs buspirone and premazepam has demonstrated a lack of sedative properties with these agents (as measured by inclined screen test, wire-grasping test, and constant rotation rotorod), and hence they have been referred to as putative nonsedative anxiolytics (PNAs). This is in contrast to benzodiazepines such as diazepam which, although anxiolytic, possesses sedative side effects.

The present study investigated the sedative properties of buspirone, premazepam, and diazepam compared to vehicle controls in female Swiss-Albino mice (N=10/drug group) using the accelerating rotorod. Mice injected with a drug were placed on the rotorod, which slowly accelerated from 4-rpm to 40-rpm over a 5-min session. The latency (sec) to fall off the rotorod was recorded. Buspirone (1.25, 2.5 and 5 mg/kg), premazepam (10 and 20 mg/kg), and diazepam (1.25, 2.5 and 5 mg/kg) all produced significantly shorter fall-off latencies than controls in a dose-dependent manner. The results suggest that the accelerating rotorod may provide a more sensitive test for measuring the sedative properties of drugs than most experimental techniques currently in use, and also question the validity of classifying buspirone and premazepam as PNAs.

547.13

EFFECTS OF MUSCARINIC COMPOUNDS ON THE FIRING RATE OF CA1 PYRAMIDAL CELLS *IN VIVO* AND ON PHOSPHOINOSITIDE TURNOVER IN HIPPOCAMPAL SLICES *IN VITRO*. R.L. Arias*, J. Cutrera*, S. Amburn*, S.M. Leventer and J.T. Haskins. Wyeth-Ayerst Research, CN 8000, Princeton, NJ 08543-8000.

Loss of cholinergic innervation to the hippocampus may contribute to the etiology of Alzheimer's disease. M1-selective agonists may therefore be of therapeutic value.

In chloral hydrate anesthetized Sprague-Dawley rats, iontophoresis of dilute solutions of acetylcholine, arecoline, carbachol and oxa-22 (5-50 nA) caused a reliable, rapid, dose-dependent increase in cell firing rate. In all cases, this effect was completely and reversibly blocked by concurrent iontophoresis of pirenzepine (5 nA). Low doses of L-glutamate also increased firing, which was only attenuated, not blocked, by pirenzepine, possibly due to blockade of septohippocampal input. Effective doses of pirenzepine alone caused a slight decrease in baseline. Interestingly, while oxa-22 and carbachol induced large increases in phosphoinositide (PI) turnover in rat hippocampal slices *in vitro*, arecoline did not. Iontophoresis of the putative M1 agonists, McN-A-343, AF102B and SR-95639A, had variable effects. At times they caused an increase in firing rate, at times a decrease in baseline or antagonism of muscarinic agonists, and at times caused no noticeable effect at all. In PI turnover, these compounds exhibited profiles consistent with partial agonism and/or antagonism.

These data indicate that (1) pyramidal cell firing rate and PI turnover are useful for assessing M1 agonism, and (2) a simple correlation between these two tests is not readily apparent.

547.14

BOTH THE α_2 ADRENERGIC AND DOPAMINE SYSTEMS CONTRIBUTE TO THE PHARMACOLOGICAL EFFECTS OF B-HT 920 IN MICE. R.L. Lloyd and M.K. Menon. Psychopharmacol. Res. Lab., V.A. Med. Ctr., Sepulveda, CA 91343 and Dept. Psychiat. UCLA Sch. Med. Los Angeles, CA 90024.

Contributions of the α_2 adrenergic and DA systems to certain pharmacological effects of B-HT 920 were investigated. Male C57 Bl/6 mice (Simonsen) were used and all injections were made i.p. The profound hypothermic response to B-HT 920 (0.1-0.5 mg/kg) was reduced by haloperiodol (0.5 mg/kg), but not by idazoxan (1.0-3.0 mg/kg). B-HT 920 (0.1-1.0 mg/kg) prolonged the duration of the ethanol (3.9 g/kg) - induced loss of righting reflex in mice. Pretreatment with idazoxan (1.0-3.0 mg/kg) fully prevented this effect of B-HT 920. B-HT 920 (0.3-1.0 mg/kg) reduced the locomotor stimulant effect of d-amphetamine in mice. Even though idazoxan (1.0-3.0 mg/kg) by itself reduced the d-amphetamine-induced hyperactivity, pretreatment with this α_2 antagonist prevented the B-HT 920-induced blockade of the amphetamine effect. It was concluded that while the hypothermic effect of B-HT 920 resulted from postsynaptic DA receptor stimulation, the other two pharmacological effects are related to its α_2-adrenergic agonistic effects (Supported by the U.S. Veterans Administration).

547.15

EXOGENOUS TYROSINE POTENTIATES THE METHYLPHENIDATE-INDUCED RELEASE OF DOPAMINE IN THE NUCLEUS ACCUMBENS AS MEASURED BY IN VIVO MICRODIALYSIS. S.K. Woods and J.S. Meyer, Dept. of Psychology, University of Mass., Amherst, MA 01003.

The synthesis and release of dopamine (DA) may, under certain conditions, be altered by increased availability of its amino acid precursor, tyrosine (TYR). To examine whether exogenously supplied TYR could potentiate the release of DA induced by methylphenidate (MPD), 7 Sprague-Dawley rats were implanted with microdialysis probes aimed at the n. accumbens. Samples were collected from awake, freely moving animals beginning 20-24 hr after surgery. Twenty-min samples were continuously collected for a 4-hr period, once a day for 3 consecutive days in a repeated measures design. On a given day, the animal was infused with 30 uM MPD, 100 uM TYR, or 30 uM MPD plus 100uM TYR in artificial CSF. Periods of infusion with the active compound(s) were preceded and followed by baseline conditions and treatments were counterbalanced to control for possible order effects. MPD plus TYR significantly increased extracellular levels of DA compared to drug alone. This effect was long-lasting, persisting into the post-treatment period and peaking 40 min after the peak induced by MPD alone. TYR alone induced a small but steady rise in extracellular DA that did not reach significance until the time of the first post-treatment sample. These results have implications for the use of TYR along with MPD in the treatment of attention deficit disorder.

547.16

ANTICONVULSANT AND PROCONVULSANT EFFECTS OF BMY 14802: RELATIONSHIP TO THE DM/SIGMA BINDING SITE. E. Echevarria, L. Robles and F. C. Tortella, Neuropharmacology Br., Div. of Neuropsychiatry, Walter Reed Army Institute of Research, Washington, DC 20307-5100.

Using two rodent models of convulsive activity we have recently shown that ligands possessing different relative affinities for PCP/sigma/dextromethorphan (DM) binding sites also profile differently in vivo (Echevarria et al., FASEB J. 4: 1990). Namely, the group I "anticonvulsant" profile includes selective high affinity PCP ligands; the group II "proconvulsant" profile includes selective high affinity sigma ligands; while the group III "anticonvulsant/proconvulsant" profile is represented by high affinity DM/sigma ligands. The purpose of this study was to investigate the effects of the putative sigma antagonist antipsychotic BMY14802 in our models: the rat supramaximal electroshock (MES; spreading convulsion) and flurothyl (seizure threshold) tests. Against MES, pretreatment with BMY14802 (25-100 mg/kg, s.c.) blocked tonic hindlimb extention (anticonvulsant ED_{50} = 50 mg/kg). In contrast, in the flurothyl test pretreatment with BMY14802 (6.25-50 mg/kg, s.c.) caused a significant lowering of the convulsive threshold. At the highest dose, this proconvulsant effect of BMY14802 approached 68% of control seizure threshold (371 vs 255 sec). Based upon these results BMY14802 appears to exhibit an in vivo profile similar to other putative DM/sigma ligands, i.e. group III as defined by our model. However, clarification of whether this drug is a functional sigma antagonist awaits additional in vivo studies.

547.17

PRETREATMENT WITH DEXTROMETHORPHAN ANTAGONIZES THE ANTICONVULSANT/PROCONVULSANT ACTIONS OF PCP/SIGMA/DM SITE LIGANDS. F. C. Tortella, E. Echevarria and L. Robles, Neuropharmacology Br., Div. of Neuropsychiatry, Walter Reed Army Institue of Research, Washington, DC 20307.

The non-opioid antitussive dextromethorphan (DM) and PCP/sigma ligands such as (+)-SKF10047 and (+)-3-PPP appear to share a common binding site but display different pharmacological profiles in vivo. We suggested that DM may be a functional antagonist at these sites. Accordingly, using an in vivo EEG and behavioral rat model, we previously demonstrated that pretreatment with DM antagonized the dual peak EEG spectral profile of (+)-SKF10047 (Tortella and Robles, Neurosci Abst. 15: 1989). In the present study, we assessed the ability of DM to alter the anticonvulsant effect of the PCP/sigma ligand (+)-SKF10047, and the proconvulsant effect of the selective sigma ligand (+)-3-PPP, in the rat flurothyl test. In this seizure threshold (ST) test, doses of DM alone up to 25 mg/kg (s.c.) were ineffective. However, pretreatment with 25 mg/kg DM significantly attenuated the (+)-SKF10047-induced increase in ST and the (+)-3-PPP-induced lowering in ST. The maximal anticonvulsant and proconvulsant effects of (+)-SKF10047 (0.125-12.5 mg/kg, s.c.) and (+)-3-PPP (12.5-50 mg/kg, s.c) were antagonized by 88% and 79%, respectively. Importantly, increasing the "agonist" dose of both (+)-SKF10047 and (+)-3-PPP two-fold to 25 mg/kg and 100 mg/kg, respectively, was sufficient to overcome the "antagonist" effects of DM, suggesting that the interaction between these ligands is competitive and reversible.

547.18

EFFECTS OF CENTRALLY-ACTING MUSCLE RELAXANTS ON ACOUSTIC STARTLE REFLEX AND MORPHINE-INDUCED RIGIDITY IN RATS. G.C. Rigdon and R.D. Harper*. Pharmacology Div., Wellcome Research Labs., Research Triangle Park, NC 27709.

This study assessed acoustic startle reflex (ASR) as a screening test for centrally-acting muscle relaxants (CMRs). ED50s were calculated for inhibition of ASR (25-msec, 120-dB white noise stimulus) and antagonism of morphine-induced rigidity (30 mg/kg morphine sulfate SC) in male, Wistar rats. Ratios of ASR ED50 to rigidity ED50 were calculated for 8 CMRs. Cinflumide, chlorzoxazone, methocarbamol, and cyclobenzaprine antagonized rigidity and inhibited ASR with roughly equivilent ED50s (ratios = 0.9 - 1.1). Diazepam and carisprodol antagonized rigidity more potently than they inhibited ASR; ratios were 1.7 and 1.9, respectively. The CMRs with ratios of 0.9 or higher are effective for the treatment of muscle spasm associated with exertion or strain. Baclofen and tizanidine (ratios 0.4 and 0.02 respectively) inhibited ASR more potently than they antagonized rigidity. These two drugs are used to treat spasticity associated with spinal cord lesion or cerebral palsy. Therefore, ASR may be helpful in determining the clinical indication for which potential CMRs are best suited.

547.19

THE DISCRIMINATIVE STIMULUS PROPERTIES OF (+)-OPIATES DO NOT GENERALIZE TO THOSE OF MORPHINE AND HAVE ANTITUSSIVE EFFECTS. P. M. Beardsley and E. W. Anthony. Dept. of CNS Diseases Research, G.D. Searle & Co., Skokie, IL 60077.

Several (+)- opiates, which do not naturally occur in nature, in addition to their naturally-occurring (-)-isomer counterparts, were evaluated in rats trained to discriminate (-)-morphine to determine if they possessed morphine's discriminative stimulus properties. Male Long-Evans hooded rats were trained to discriminate 3.0 mg/kg s.c. (-)-morphine from water, vehicle, while lever pressing under fixed ratio 10 reinforcement schedules for food pellet delivery. Following discrimination training, dose-response functions for (-)-morphine were characterized. Subsequently, generalization tests with (+)-morphine, (+)- and (-)-heroin, (+)- and (-)-etorphine, (+)- and (-)-nordihydrothebainone, and (+)- and (-)-dihydrothebainone were conducted. The (-)- isomers of morphine, heroin, etorphine, and dihydrothebainone all generalized to the morphine discriminative stimulus in a dose-dependent fashion and induced greater that 90% morphine-lever responding at least at one dose tested. The order of potency for generalizing to the morphine stimulus was: (-)-etorphine>>(-)-heroin>(-)-dihydrothebainone=(-)-morphine. The (-)- isomer of nordihydrothebainone did not generate more than 25% morphine-lever responding up to 30 mg/kg, the highest dose tested. At doses of the (-)-opiates that did generalize to the morphine stimulus response rates were often reduced relative to vehicle-control levels. None of the (+)-isomers generalized to the morphine stimulus and only (+)-nordihydrothebainone had marked effects on response rates. In other studies it was found that the (+)-isomers of morphine, etorphine, heroin, codeine, dihydrocodeine, and dihydromorphine possessed antitussive activity when evaluated in the citric acid-induced coughing reflex test in the guinea pig. These results suggest that some of the (+)-isomers of the opioid alkaloids could be free of morphine's subjective effects, and thus possibly its abuse liability, while having therapeutic utility as antitussives.

NEUROETHOLOGY: FISH

548.1

SENSORY GUIDANCE OF PASSIVE ELECTROLOCATION: A STATIC AND A DYNAMIC ANALYSIS. C.D. Hopkins, D. McBride*, K-T. Shieh* and G.H. Harned*, Section of Neurobiology and Behavior, Cornell University, Ithaca, NY 14853.

South American electric fish (Gymnotiformes) make well directed approaches toward conspecifics or toward electrodes playing conspecific signals. We explored how electrosensory cues guide this "passive electrolocation" by observing, under infrared light, the approach responses to electric fields from dipoles. Behavioral trials were recorded on a video recorder, and analyzed frame-by-frame. *Gymnotus carapo* orients its body axis parallel to the local electric field while swimming toward the source, either forward or backward. We compared both the paths and the postures of the fish to the direction of the local electric field, and we computed an "error" angle between the fish's direction and the electric field. The direction of the electric field was determined by computation using the method of images to account for the effects of the tank boundary.

The error angle was consistently 0° or 180° for three electrode geometries. All parts of the body were similarly aligned, but the head showed the greatest variance around 0°. Even when the electrodes rotate at speeds up to 50 rpm, the fish continues to align to the electric field.

These fish frequently "err" to the left or the right of the electric field direction, but correct their error with a 0.2 to 0.5 s lag time, by bending the body in the appropriate direction. Fish that experience a rapidly-changing error angle may stop and reverse their direction in a V-turn. From these behavioral observations we conclude that the fish has an accurate sense of the direction of the local electric field through its body, and that this directional sense guides subsequent turning behavior with a short delay. Supported by NIMH grant # R01 MH 37972 and NSF grant # BNS 8810080.

548.2

DIRECTIONAL CHARACTERISTICS OF TUBEROUS ELECTRORECEPTORS IN THE WEAKLY ELECTRIC FISH, *HYPOPOMUS* SP. D.D. Yager and C.D. Hopkins, Section of Neurobiology and Behavior, Cornell University, Ithaca, NY 14853

Pulse type electric fish are able to localize the source of an electric field, such as that produced by a conspecific, by aligning their long body axis parallel to the local electric field and following the current lines until they reach the source. We are interested in the sensory cues that the fish could use to determine its alignment error. We recorded from > 150 afferents from tuberous electroreceptors in the ganglion of the anterior lateral line nerve in fish suspended at the center of a large circular tank. Stimuli mimicking the electric organ discharge (EOD) were presented through pairs of electrodes spaced at 15° intervals around the tank perimeter. We precisely located each receptor on the body surface using a monopolar probe.

Variations in stimulus angle cause systematic variations in the responses of both burst duration coder and pulse marker afferents. Polar plots of spike number, latency, and threshold all have two elliptical lobes oriented 180° apart. The lobes are often markedly asymmetrical with the major lobe pointed into the fish. The "best" angle varies systematically over the body surface with pulse markers and burst duration coders in the same area showing similar preferences. Units on the snout, tail, and dorsal midline prefer fields parallel to the body axis, while units located on the flank, especially over the thickest part of the body, respond best to transverse fields. The greatest overall sensitivity to any stimulus angle is found on the head; the lowest is found on the tail.

Our data establishes that information is available to the CNS to permit left versus right and head versus tail comparisons as a possible basis for correcting orientation errors. Supported by NIMH grant # R01 MH 37972 and NSF grant # BNS 8810080.

548.3

BEHAVIORAL TEST OF LATENCY SHIFT DETECTION IN MORMYRID ELECTRIC FISH. R. Zelick and C.C. Bell, Portland State Univ., Portland, OR 97207 and R.S. Dow Neurol. Sci. Inst., Portland, OR 97209.

African weakly electric fish (Mormyridae) possess three separate electrosensory systems. One of these, the mormyromast system, is adapted for detection of modulations in the fish's electric field caused by objects in the environment. Over a behaviorally relevant range of electric field intensities, primary mormyromast afferents produce only one to several spikes, but vary smoothly and monotonically in latency relative to the fish's electric organ discharge (EOD) or to the motor command signal that drives the EOD (Bell, C.C., *J. Neurophysiol.*, 63:319, 1990). This suggests that spike latency, rather than spike rate, may code for intensity modulations of the electric field. Therefore, a series of behavioral experiments was undertaken to test the sensitivity of the fish to shifts in electric field latency relative to the EOD command signal.

Latency sensitivity measurements were made on three species of mormyrids (*Brienomyrus niger, Gnathonemus petersii, Mormyrus rume*). Individuals were curarized and presented with computer generated and timed mimics of the fish's natural EOD. Latency timing was randomized between presentations. The novelty response, a transient acceleration of command signal rate, was the behavioral response measured.

Latency shifts of -2 ms were detected by the fish only over a range of latencies from 2 to12 ms following the command signal, with the greatest response at 4 ms latency. At a fixed latency of 2 ms, shifts greater than ± 0.5 ms were easily detected. This range of behavioral sensitivities correlates favorably with the responses of primary mormyromast afferents and supports the notion of a latency code for stimulus intensity. SUPPORTED BY BNS 8810571.

548.4

CELLS SENSITIVE TO TIME-DOMAIN INFORMATION IN THE MIDBRAIN OF MORMYRID ELECTRIC FISH. S.Amagai and C.D.Hopkins. Neurobiology & Behavior, Cornell University, Ithaca, NY 14853

Mormyrid electric fish are capable of recognizing brief externally generated pulsatile electrical stimuli by reference to the temporal, not spectral characteristics of the electrical waveform, with an acuity in submillisecond range (Hopkins & Bass *Science* 212: 85-87 1981). The Knollenorgan electroreceptor system is thought to mediate this behavior; it sends timing information from peripheral receptors to the toral nucleus exterolateralis pars anterior (ELa) via a rapidly conducting pathway. From there it projects to the adjacent nucleus exterolateralis pars posterior (ELp). A precise phase-locking is maintained up to ELa but disappears in ELp. Here we report findings on the properties of ELp using field and single-unit recordings.

Field potentials recorded from ELp revealed that a single outside positive voltage step is sufficient to elicit a large slow field potential with a latency of 6-8ms. Square waves of varying duration were used to simulate different waveforms having distinct temporal characteristics, but we could find no detectable differences in field potentials that is not predicted from the simple convergence of peripheral inputs at ELp.

Most single units recorded in ELp showed little or no spontaneous activity but units fire spikes in response to external square waves and steps. There is little evidence of the precise phase-locking evident in ELa, but rather, ELp cells seem to respond sporadically with a variable number of spikes ranging from one to about 10. Our preliminary findings suggest that there are at least two types of cells in ELp. First, there are cells which respond to unilateral stimulation of a specific body surface area with a latency of 8ms. They show no appreciable change in response when the stimulus duration or polarity is changed. Second, there are cells which are tuned to a specific duration of square wave stimuli. These temporally tuned cells are also polarity sensitive, have a longer latency (12ms) and often fire more spikes per burst compared to the other cell-type. Such sensitivity to temporal disparity of activation in different areas of the body can be used for recognition of the stimulus waveform. Supported by NIMH grant MH37972 to CDH.

548.5

CELL TYPES AND MODIFIABLE COROLLARY DISCHARGE SIGNALS IN THE MORMYROMAST REGION OF THE ELECTROSENSORY LOBE OF MORMYRID ELECTRIC FISH. C.C. Bell and K. Grant, R.S. Dow Neurol. Sci. Inst., Portland, OR 97209 and Lab. de Physiol. Nerv., C.N.R.S., Gif sur Yvette, 91190, France.

Mormyromast electroreceptors are the most numerous type of electroreceptor in mormyrid fish and are responsible for active electrolocation. Little is known, however, about the central processing of information from these electroreceptors. Single cells were therefore recorded in the mormyromast region of the electrosensory lobe, the first central relay of the system. The effects of local electrosensory stimuli to the skin and of the electric organ discharge (EOD) motor command were examined with extracellular recording.

Three major categories of cells were found: 1) cells excited by the EOD command and inhibited by electrosensory stimuli; 2) cells in which the EOD command had only moderate effects in isolation but strongly facilitated an excitatory response to a stimulus; 3) cells inhibited by the EOD command and excited by electrosensory stimuli. Distinct subtypes were present within the first two categories.

The effects of the EOD motor command were modifiable and depended on previous pairing of the command with a sensory stimulus. The direction of modification was to oppose the effect of the paired stimulus. Two types of modification in command effect were evident; a large modification occurring over several minutes, and a small modification occurring over a few seconds. The slow modification is similar to that described previously in the ampullary region of the electrosensory lobe and could serve in adjusting the system to slow environmental changes. The fast modification was not observed previously and could serve in the active electrolocation of rapidly changing events.

548.6

ELECTROSENSORY MODULATION OF ESCAPE. J. G. Canfield and G. J. Rose, Dept. of Biology, Univ. of Utah, Salt Lake City, Utah 84112.

Using a sudden-onset pressure stimulus, we show that the weakly electric fish Eigenmannia maintains a low threshold to startle, demonstrates a diverse array of escape responses, and easily avoids obstacles regardless of ambient light levels. These fish also suppress escape maneuvers through an electric field mimicking the presence of a conspecific.

In contrast, the highly visual goldfish, Carassius auratus, easily avoid obstacles and have a diverse range of maneuvers only when pressure-startled in a well lighted aquarium, and usually when the animal is actively swimming about the tank. Escape rarely occurs when goldfish are in a "freezing" position. In dim light, escape responses are rare, but obstacles are again avoided, though the magnitude and trajectory of the responses are highly restricted. (See also Emberly & Eaton, *Soc. Neurosci. Abstr.*, 1990) "Freezing" behavior is an ethologically important strategy to visually-oriented prey for maintaining concealment and optimal detection of predators.

Physiological evidence obtained from Eigenmannia support the above behavioral data. While the Mauthner cell does not appear to receive electrosensory inputs directly, such inputs have been recorded in other reticular formation cells. The spike activity of these cells is influenced by a mimic of the fish's own electric organ discharge. These putative "non-Mauthner" escape-modulating brainstem neurons (Eaton et al., *Brain, Behav. &Evol.*, in press) may be specific to weakly electric fish. The ability of Eigenmannia to maintain robust escape responses and avoid obstacles in a nocturnal environment appears to be related to its electrosensory abilities. Supported by NSF and the Sloan Foundation.

548.7

DISCRIMINATION OF THE SIGN OF FREQUENCY DIFFERENCES BY THE WEAKLY ELECTRIC FISH, STERNOPYGUS. G.J. Rose and J.G. Canfield. Dept. of Biology, Univ. of Utah, Salt Lake City, UT 84112.

In its 'Jamming Avoidance Response', the gymnotiform electric fish, Eigenmannia, shifts the frequency of its electric organ discharges (EODs) away from that of a 'jamming' signal. The fish is able to correctly determine the 'sign' of the frequency difference (Df) between its EODs and those of the other fish. Behavioral and neurophysiological studies indicate that Eigenmannia determines the sign of Df by a complex analysis of the temporal relationship between modulations of the amplitude and phase of signals received by different areas of the body surface.

To gain insight into the evolution of this behavior, studies were conducted on fish of the related genus, Sternopygus. An associative conditioning paradigm was developed to determine if Sternopygus, despite lacking a JAR, is able to discriminate the sign of Df. Movement of a 'shuttle' in one direction was preceded by presentation of a signal slightly higher in frequency than that of the fish's EOD, while a signal slightly lower in frequency was paired with the opposite direction of movement. Both species of Sternopygus were able to learn this discrimination within 1000 trials. Evidence will be presented supporting the notion that these fish achieve this discrimination by the same mechanism as does Eigenmannia and that they possess modulation filters. Supported by grants from NSF and Sloan Foundation.

548.8

NEURONAL CODING AND PROCESSING OF COMMUNICATORY SIGNALS IN THE ELECTRIC FISH, EIGENMANNIA. W. Metzner * and W. Heiligenberg. SIO, UCSD, La Jolla, CA 92093

Eigenmannia produces continual, nearly sinusoidal electric organ discharges (EODs) and has two classes of electroreceptors, ampullary units which code low-frequency signals (0 - 40Hz), and tuberous units which are tuned to the fish's fundamental EOD frequency (200 - 500Hz). Separate structures process the inputs from these two receptor classes in the electrosensory lateral line lobe (ELL) of the hindbrain and project to overlapping targets in the torus semicircularis of the midbrain, which in turn projects to the nucleus electrosensorius (nE) of the diencephalon. A regular courtship and aggressive signal are brief interruptions of the EOD, or 'chirps'. The low spectral frequencies of chirps stimulate the ampullary system, whereas fast transients excite the tuberous system. Another social signal are 'beats', phase and amplitude modulations of the electric signal which result from the interference of different EODs of similar frequencies. Beats, which lack low spectral frequencies, are coded by tuberous receptors only and control the fish's jamming avoidance response (JAR). Up to the level of the nE, responses to chirps become more distinct, and neurons are excited more selectively by either chirps or beats. A pathway of chirp-sensitive neurons leads from the nE to the inferior lobe and may affect the pituitary.

548.9

DIFFERENT CLASSES OF GLUTAMATE RECEPTORS MEDIATE DISTINCT MODULATIONS OF THE MEDULLARY PACEMAKER OF A GYMNOTIFORM ELECTRIC FISH. Masashi Kawasaki AND Walter Heiligenberg. UCSD, La Jolla, CA 92093

Gymnotiform electric fish generate distinct communicatory signals by modulating the rate of their electric organ discharges (EODs). Each EOD is triggered by a command pulse from the medullary pacemaker nucleus which contains pacemaker cells and relay cells. The firing rate of this nucleus is modulated by inputs from the diencephalic prepacemaker nucleus. The pharmacological separation of behaviors previously found in the 'wave' genus Eigenmannia has been confirmed in the 'pulse' genus Hypopomus. The NMDA receptor blocker APV and the kainate/quisqualate receptor blocker CNQX, administered to the pacemaker nucleus, suppress the frequency rises and 'chirps', respectively, indicating that different classes of glutamate receptors mediate the generation of different modulations. In Hypopomus, a form of sustained modulations, the 'sudden interruption', appears to be mediated by NMDA receptors as well. Despite the rather different frequencies of electric organ discharges observed in these genera, it appears to be a common feature that NMDA receptors are used for sustained modulations, whereas kainate/quisqualate receptors mediate rapid modulations.

548.10

CONDUCTANCES CONTRIBUTING TO THE ACTION POTENTIAL WAVEFORM OF ELECTROCYTES IN *STERNOPYGUS*. M.B. Ferrari and H.H. Zakon. Dept. of Zoology, Univ. of Texas, Austin, Texas, 78712.

Electrocyte spike duration (ESD) in this species varies with the electric organ discharge frequency. The ESD is longer in males than in females and can be modulated by steroid hormones. A current clamp study was used to investigate membrane properties using blockers and ion substitutions to determine some of the conductances (g's) which contribute to the shaping of the spike waveform (SPWF). The average resting potential was -84.9 +/- 9.8mV in cells with ESDs ranging from 3.45 to 11.6ms (n=62). The average overshoot amp. was 18.75 +/- 16.59mV (n=41). A complete but reversible block of the spike occured in 1.25uM TTX. In addition, AS-II, which blocks sodium inactivation, resulted in a dramatic increase in both ESD and amp. (5.05 to 14.35ms and +17mV amp in one case). Ca++ g's, however, do not appear to contribute significantly to SPWF as both 0 Ca++ and 500uM - 2mM Co++ do not alter SPWF or IV curves. These results also suggest that Ca++-activated K+ g's are not involved in shaping SPWF (this is further supported as 1uM apamin had no effect). Both the rising and falling phases of the spike appear to be shaped by K+ g's. Application of 20 - 40mM TEA prolonged the falling phase and resulted in a slightly faster rise time and increase in amp. The TEA effects were also reversible. The application of 1-5mM 4-AP, however, caused a more dramatic increase in spike amp. and resulted in an even faster rise time than that observed with TEA. The effect of 4-AP on the falling phase also appeared qualitatively different than that of TEA. These results suggest the presence of at least two separate K+ g's. An increase in g during hyperpol. was also blocked by 4-AP. In twin-pulse expts., a hyperpol. pre-pulse results in a spike onset delay during the depol. pulse, implicating an A-type cond. and/or an inward rectifier in shaping the rising phase of the spike. The results to date are consistent with a Na+ spike which is shaped by the interplay of at least 3 K+ g's: an inward rectifier, an A-type g, and a delayed rectifier. Supported by NIH.

548.11

BROMODEOXYURIDINE LABELLING OF REGENERATING ELECTRIC ORGAN REVEALS A CLASS OF SATELLITE-LIKE CELLS. J.M. Patterson, and H.H.Zakon. Dept. of Zoology, University of Texas at Austin, Austin Tx., 78712.

Weakly electric fish of South America, the gymnotiforms, are a highly regenerative vertebrate species. Many species regenerate their posterior body parts, including muscle, nerve, and electrocyte cells (EC) of the electric organ (EO). In *Sternopygus*, the EO is made up of long (1000 µm), cylindrical cells which have been reported as having a myogenic origin. EM micrographs of EO reveal small satellite-like cells around the periphery of the EC embedded in the extracellular matrix. We have studied these cells using the bromodeoxyuridine (BRDU) labelling technique and found that they divide and proliferate after EO amputation.

Nine *Sternopygus marcrurus* had a 10-20 mm segment of the tail posterior to the anal fin removed, and were injected with either 50 mg/kg BRDU or saline after 2, 4, and 6 days. After a 3 hr. labelling period, the proximal stump was fixed, frozen sections were cut, incubated with anti-BRDU Ab, and visualized with fluorescein or HRP-conjugated mouse secondary Ab.

Two days post-amputation (n=3), satellite-like cells close to the cut were labelled, as well as epidermal cells. At day 4 (n=3) there is extensive labelling of satellite-like cells near the wound margin, and at sites several hundred microns proximal to the cut. We observe an aggregation of rapidly dividing cells near the stump, indicative of a regeneration blastema. After 6 days of regeneration (n=3), labelled satellite-like cells occur throughout the stump, and a large blastema containing many labelled cells is present. No labelling was seen in any saline-treated control animals. These results suggest there may be a previously unreported pool of satellite cells within the electric organ, and that division of these cells may contribute to the formation of the regeneration blastema. Supported by NIH.

548.12

ELECTROSENSORY LATERAL LINE LOBE EFFERENTS: A MORPHOLOGICALLY DISTINCT CELL TYPE PROJECTS TO THE CAUDAL N. PRAEEMINENTIALIS OF WEAKLY ELECTRIC FISH. J. Bastian and J. Courtright*. Dept. of Zoology, Univ. of Oklahoma, Norman, OK 73019.

The electrosensory lateral line lobe (ELL) is the initial CNS processing area for the electric sense and previously two categories of electric organ discharge (EOD) amplitude encoding neurons have been described. The basilar and non-basilar pyramidal cells of the ELL respond to increases in EOD amplitude with predominantly phasic increases and decreases in activity respectively. These cells project to the torus semicircularis and the n. praeeminentialis (NP). Recent studies of the NP identified neurons which encode EOD amplitude tonically. Afferent terminals were also identified within the NP having tonic response properties. These results suggest that another category of ELL output neurons exists. Extracellular injections of HRP into the caudal NP retrogradely label a morphologically distinct ELL neuron type. These fusiform cells are found in the ELL granule cell layer, have somas that are smaller than those of the basilar and nonbasilar cells, and these ELL neurons project bilaterally to both the ipsi- and contralateral NPs. All of the retrogradely labelled deep cells had a basilar dendrite extending into the electroreceptor afferent fiber layer, and the apical dendrites of these neurons differed markedly from those of the previously described ELL output cells. Their apical dendrites are short, extending dorsally through about 50% of the ELL molecular layers and they are sparsely branched, whereas the apical dendrites of the basilar and nonbasilar cells branch extensively and extend through the entire dorsoventral extent of the ELL molecular layers. Physiological experiments are underway to determine if these deep ELL neurons provide the tonic measures of EOD amplitude seen within the n. praeeminentialis. Supported by NIH grant NS12337 and OCAST contract # 1669.

548.13

PHASE AND AMPLITUDE MEASUREMENTS OF THE ELECTRIC ORGAN DISCHARGE OF *APTERONOTUS LEPTORHYNCHUS*. C. Assad, B. Rasnow, J.M. Bower. Depts. of Elect. Eng., Physics, and Biology, Caltech, Pasadena, CA 91125.

A. leptorhynchus is a weakly electric gymnotiform fish with a high frequency (600 to 1000 Hz) wave-type electric organ discharge (EOD). Previous measurements of the EOD in the midplane distant from the fish have shown an approximate dipole field with poles centered in the trunk and tail. In the current experiments, we have mapped the electric potential and electric field components of the EOD adjacent to the skin at numerous horizontal and vertical positions over one side of the fish. Recordings from different positions were synchronized by an EOD phase reference measured at a stationary electrode, which allowed the precise temporal structure of the EOD to be determined. Electric field components normal to the fish's skin were calculated. Results reveal a more complex EOD portrait than that seen with far field measurements, especially in the tail region where complex phase relationships were found.

These results provide important constraints for our efforts to model both the generation of the EOD and the central analysis of electrosensory data. With respect to the electric organ, our data suggests the electrocytes do not fire synchronously. But the stability of the EOD waveform over multiple periods suggests that the relative phase of firing of these electrocytes is tightly controlled. With respect to the processing of electrosensory information, our results indicate that the tuberous electroreceptors found in the tail region experience a considerably different electrical environment than those in the trunk and head. In addition to complex phase relationships, field amplitudes near the tail are an order of magnitude larger than over the trunk. Since the sensory field is highly sensitive to tail position, the fish must either *control* this parameter precisely, and/or *compute* its effects. These results suggest that information from the caudal electroreceptors may be used differently in central processing.

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548.14

THE DORSAL FILAMENT OF THE WEAKLY ELECTRIC APTERONOTIDS IS SPECIALIZED FOR ELECTRORECEPTION. C. Franchina*, C.D. Hopkins, and A. Schneiderman, Section of Neurobiology and Behavior, Cornell University, Ithaca, New York 14853.

Among the Gymnotiforms, weakly electric South American fish, only members of the Apteronotid family possess a dorsal filament (or thong). The filament is a long semicylindrical structure which lies in a shallow groove on the back and whose function is unknown. We investigated the gross anatomy of the filament in eight genera (fourteen species) of Apteronotids, and the cellular anatomy of the filament in one species of *Adontosternarchus*. We conclude that the dorsal filament functions primarily in electroreception.

We examined preserved specimens of Apteronotids collected in Venezuela and fresh tissue samples from euthanized animals which were fixed, embedded in paraplast, and sectioned at 6-20 µm. Sections were stained for general features (Mallory's trichrome), PAS+ elements (Schiff reaction), or neurofilaments (Bodian's silver stain).

The anterior origin of the filament varied in position from 0.64 body lengths behind the head (in *Sternarchorhynchus*) to 0.49 body lengths behind the head (in *Sternarchogiton*). The filament is a long thin structure, wedge-shaped in cross-section, and continuous with the body of the fish only at the anterior end. The filament and the groove in which it lies are covered by separate sheets of pigmented, unscaled epidermis, but are sealed together by a mucosal layer in living animals. The exposed dorsal surface of the filament is covered with tuberous electroreceptors whose heavily myelinated afferents join two nerves on the ventral margin. Blood vessels are also located ventrally. The bulk of the filament is a mucopolysaccharide matrix.

We speculate that the dorsal filament of the Apteronotidae may be homologous to the adipose fin of the Siluriformes and Characodei. The filament is ideally situated to detect the animal's own electric organ discharge without contamination from other signals. (Supported by A.D. White Fellowship to C.F. and NIMH Grant MH 37972 to C.D.H.)

548.15

AUDITORY NEUROPHYSIOLOGY OF THE MESENCEPHALON IN MORMYRID FISH. J.D. Crawford & R.R. Fay*. Parmly Hearing Institute, Loyola U., 6525 N. Sheridan Rd, Chicago, IL 60626.

We are interested in temporal processing and the neural representation of complex sounds, and are studying mormyrids because they use temporally-patterned sounds in communication (Crawford et al. 1986), they have peripheral gas bladders for sound pressure transduction at the sacculi, and their auditory pathways are known anatomically. Here we summarize responses of single neurons, in nucleus Medialis Dorsalis (nMD), stimulated with tones and clicks presented underwater.

We encountered neurons that gave either phasic responses or tonic responses to tones. Most had low spontaneous activity ($\leq$ 1.0 spikes/s) and responded with short latencies (5 ms). Typical thresholds were -30 to -15 dB r.e. 1.0 dyne/cm^2 (communication signals are +30 dB at 10 cm). Characteristic frequencies (CFs) of tonic neurons were at about 235 Hz and this is very close to the 220 Hz fundamental of a nearly tonal signal produced during courtship. Some of these tonic neurons exhibited sharp frequency selectivity ($Q_{10dB} \geq 5$). The CFs of phasic neurons have also been primarily in the 235 Hz range but some had higher CFs between 400 & 500 Hz and all were broadly tuned ($Q_{10dB} \leq 1.0$).

All phasic neurons showed tight synchronization to clicks during trains with intervals from 10-60 ms, a range that included intervals characteristic of two pulsatile sounds also used in communication. Many nMD neurons had non-monotonic rate-intensity functions with a peak at about +5 dB. We have also observed phasic units that gave both onset responses and *off-responses* 40 ms after tone bursts, but only at relatively high levels (e.g. 20 dB). We are currently investigating the possible role of inhibition in bringing about these nMD features.

These response properties are consistent with the idea that nMD functions in processing the tonal and click type sounds used in mormyrid communication (NIH NRSA DC00020-02 & CDR P50 DC00293-06).

548.16

LHRH-POSITIVE PREOPTIC CELLS IN A SEX REVERSING FISH: SEXUAL POLYMORPHISM AND MODULATION BY GONADAL STEROID HORMONES. M.S. Grober and A. Bass . Section of Neurobiology and Behavior, Cornell University, Ithaca, N.Y. 14853.

The sex-reversing fish, *Thalassoma bifasciatum*, exhibits two color morphs (primary phase, PP, and terminal phase, TP). PP fish are born either male or female, while TP males result from the transformation of PP males or females. Previously, we identified LHRH-like immunoreactive cells in the preoptic area (POA) of this species. This analysis provides a quantitative comparison of LHRH POA cells amongst the different sexual phases and addresses the role of gonadal steroid hormones in regulating cell size and number.

Total LHRH POA cell number was 2-3 times higher in TP males versus PP males or females, which were similar to each other. There was no significant difference in the size of LHRH POA cells between any of the sexual phases. For both PP males and females, intraperitoneal implants of 11 Ketotestosterone, a naturally occurring gonadal androgen, induced significant increases in the number of LHRH POA cells to levels observed in untreated TP males. There was, however, no significant effect of androgen implants on either cell number in TP males, or cell size in any of the sexual phases. Finally, all steroid treated PP fish assumed TP coloration. These results demonstrate a naturally occurring sexual polymorphism in LHRH POA cell number whose expression is influenced by gonadal steroid hormones. Given the known role of the POA in the control of reproductive function, LHRH POA cell number may in fact determine the differences in reproductive behavior that are characteristic of the different sexual phases in sex reversing fishes. Supported by a NIH Postdoctoral Training Grant (MSG) and NIH / NSF Grants (AB).

548.17

EFFECT OF SIZE AND SEX ON GRUNT PRODUCTION IN THE OYSTER TOADFISH. T.D. Waybright*, U. Kollenkirchen* and M.L. Fine. Dept. Biology, Va. Commonwealth Univ., Richmond, VA. 23284-2012.

Male and female toadfish (Opsanus tau) produce an agonistic grunt call by contracting a pair of sonic muscles intrinsic to the swim bladder. The swim bladder and sonic muscles increase in size for life and are both larger in males than in females. In this study we investigated the effect of fish size and sex on spontaneous grunts recorded in air. Fundamental frequency ranged between 120 and 160 Hz and did not vary with fish size or sex, confirming that the frequency spectrum is determined by muscle contraction rate and not swim bladder size. Individual grunt trains varied from 3 to more than 30 grunts, and grunt durations varied from 13 to over 200 ms. In a long series, durations tended to decrease and intergrunt intervals to increase with time. Although all fish were recorded under identical conditions, SPLs within a train ranged between 3-13 dB and were similar in males and females. Although the toadfish sonic system is thought of as all-or-none, this variation suggests that the fish can recruit different numbers of motor units. Maximum SPLs were approximately 70 dB re. 20uPa at 20 cm in males and females and tended to increase with fish size.

548.18

EMG AND KINEMATIC ANALYSIS OF THE STAGES OF THE MAUTHNER-INITIATED ESCAPE RESPONSE. M. B. Foreman and R. C. Eaton. Center for Neuroscience, University of Colorado, Boulder, CO 80309-0334.

We are using simultaneous electromyographic (EMG) recording and high speed image acquisition and analysis to understand the neural basis of the Mauthner initiated C-start escape of the goldfish. The initial biomechanical component, stage 1, is a sudden bending of the body whereas stage 2 is a forward acceleration that can also include a turn. Previously we reported results in which some of the kinematic parameters of the C-start were related to the underlying muscular activity based on EMG records. Here, we extend this analysis based on refinements in image and EMG processing. We report our findings based on seven strictly quantified kinematic descriptors that characterize the progression of the behavior.

Of the many correlations between the EMG and kinematic characters, of special interest is the fact that the rectified integral (volume) and duration of the stage 1 EMG burst are correlated with linear velocity and distance covered by the fish in stage 2. This suggests variable motoneuron recruitment during stage 1. Moreover, the duration of stage 1 movement is proportional to the stage 2 EMG burst latency. From this we postulate that the command underlying the stage 2 contraction serves to terminate stage 1. If this is the case, then neurons triggering stage 2 are coded by the angle of the impinging stimulus. [Supported by NIH grant NS22621].

548.19

SENSORIMOTOR COORDINATION BETWEEN ATTACK ANGLE AND THE STAGES OF THE MAUTHNER-INITIATED ESCAPE RESPONSE. D. S. Emberley* and R. C. Eaton. Center for Neuroscience, University of Colorado, Boulder, CO 80309-0334.

We are studying the Mauthner initiated C-start escape of the goldfish as a neuroethological model for sensorimotor coordination. This escape behavior has two biomechanical stages. Stage 1 is an initial bending of the body whereas stage 2 is a forward acceleration caused by a stroke of the tail. Stage 2 can also include a turn. To emulate predatory attacks, we dropped a ball into the water above the fish and we measured the relationship between the angle of attack and the stage 1 and 2 turns. We found that the attack angle reliably predicts these turns. For stimuli behind the fish, stages 1 and 2 are small such that the animal accelerates forward in the direction of its initial orientation. To avoid stimuli in front, stages 1 and 2 are large so that the animal reverses its initial orientation. Thus, the direction of the stimulus can code the neural commands controlling stage 1 and 2 muscle contractions. In addition, nearby obstacles can modulate the command and alter the escape route. In cases when the fish made an error and turned toward the stimulus, there was no trajectory correction. Thus, once generated, the neural commands are resistant to subsequent sensory modulation even though such errors might increase the probability of capture. [Supported by NIH grant NS22621].

548.20

QUANTITATIVE AUTORADIOGRAPHIC STUDIES OF T_3 AND IGF RECEPTOR BINDING AND PROTEIN SYNTHESIS IN THE BRAINS AND PINEAL OF COHO SALMON DURING SMOLT TRANSFORMATION

Sven O.E. Ebbesson[1], Thomas Östholm[1]*, Dennis Baskin[2], Lars O.E. Ebbesson[1]*, and Lawrence Duffy[1].

[1]Institutes of Marine Science and Arctic Biology, University of Alaska Fairbanks Fairbanks, AK 99775 and [2]Department of Medicine and Biological Structure, University of Washington, Seattle, WA 98108

Smolt transformation (ST) occurs midlife in coho salmon. During this brief period coho salmon imprint on olfactory cues of their natal stream and change behavior. ST is associated with a plasma thyroxine surge and our recent studies on the brains have revealed sequential surges in brain content of some monoamine and amino acid neurotransmitters, changes in immunoreactivity of substance P and FMRF amide in addition to extensive expansion of some axonal systems. In the search for factors controlling or affecting the neural events associated with ST we have employed standard methods for quantitative autoradiography of T_3 (*in vivo*) and IGF (*in vitro*) receptor binding and methionine (^{35}S) incorporation in salmon before, during and after ST. All three studies revealed significantly higher levels of radioactivity in the pineal than in any part of the brain. IGF receptor binding in the pineal and habenula was high in the three salmon populations while the olfactory bulb in the smolt exhibited greater binding than in presmolts and postsmolts. The telencephalon in the smolt also showed greater binding than in pre- or post smolts. Protein synthesis in the pineal, as measured by ^{35}S labeled methionine incorporation, was exceptionally high in the three populations and in the olfactory bulb and telencephalon synthesis decreased in smolts and post smolts. These results indicate that the pineal may play a significant role in ST and that the neural plasticity associated with it involves complex interactions of several hormonal and neurotransmitter systems.

Supported by research grants from N.I.H., Sea Grant and the V.A.

548.21

LATERALIZED ESCAPE BEHAVIOR IN HEMISPHERICALLY ASYMMETRIC SALMONIDS. P. Ahluwahlia and G.L. Chew*. Psychology Dept., University of Lethbridge, Lethbridge, AB, Canada, T1K 3M4.

The escape behavior of rainbow trout (Oncorhynchus mykiss) and coho salmon (O. kisutch) (age approx 6 months) was found to be lateralized. That is, individual fish showed consistent preferences in the direction in which they swam to shelter upon release from entrapment. Moreover, different populations of trout and salmon were found to be differentially lateralized: some populations showed sinistral preferences whereas others showed dextral preferences.

As a first step in understanding the neural bases of these behavioral asymmetries we characterized the gross anatomical and volumetric properties of various discrete brain structures of left- and right-escaping fish. We found structural asymmetries, particularly in telencephalic regions, which paralleled the observed lateralization in escape behavior.

549.1

MIF-1 IMPROVES RETENTION PERFORMANCE OF AGED RATS IN AN ACTIVE AVOIDANCE PARADIGM. G. A. Olson, A. S. Wensel*, A. J. Kastin, M. C. Brown*, and R. D. Olson. Department of Psychology, University of New Orleans, New Orleans, LA 70148

As part of our program evaluating the Tyr-MIF-1 family of endogenous opiate antagonists, 24-month old male albino rats were injected IP with either morphine (1.0 mg/kg), MIF-1 (Pro-Leu-Gly-NH_2, 0.1 mg/Kg), or the diluent vehicle. Animals were injected immediately after Day I training and 11 days later 10 min before Day II retention testing. In both sessions, the animal was placed in an automated shuttle-box where the CS was a tone-light combination, the US was 0.5 mA of shock, and the CS-US interval was 10 sec. Trials were initiated on a VI-60" schedule. Testing continued on each day until the animal reached the criterion of 8 avoidances over the last 10 trials.

Results indicated that post-training injections did not yield any significant retention differences. The pre-retention injections with MIF-1, however, significantly reduced the number of avoidance responses required to reach criterion and decreased the response latenices from trial 1 to trial 2.

The results suggest that MIF-1 may play a role in facilitating performance of a previously learned active avoidance response in aged rats.

549.2

EFFECTS OF CORTICOSTEROIDS ON MEMORY IN HUMAN AMNESIA: A NEUROPSYCHOLOGIC CASE STUDY R.J. Caselli, M.R. Trenerry*. Departments of Neurology and Psychiatry, Mayo Clinic, Rochester, MN 55905.

A 41 year old amnesic man with bilateral hippocampal infarctions (larger on the right) underwent serial neuropsychological testing before, during, and following corticosteroid administration (prednisone 30 mg/day) to explore the hypothesis that steroids potentiate hippocampal contributions to memory. Effects were modest, but short term retrieval of noncontextual verbal material and delayed retrieval of contextual verbal material were improved on steroids. Visual memory showed no appreciable improvement. Test-retest effects were mild, except for marked improvement on the Wisconsin Card Sorting Test. No improvements were noted on tests of a variety of non-memory functions, including attention and psychomotor speed. We conclude that corticosteroids may have a modest, specific beneficial effect on certain aspects of declarative memory, independent of any effects on attention and psychomotor speed.

549.3

GALANIN PREVENTS RETENTION OF ONE-TRIAL REWARD LEARNING. D.H. MALIN, J.F. NOCK*, B.J. NOVY*, J.R. LAKE, R.E. PLOTNER*, A. LETT BROWN*, L.M. ARNDT* AND L.D. OSGOOD*. Univ. of Houston-Clear Lake, Houston, TX 77058.

Galanin is a neuropeptide that coexists with acetylcholine in the septohippocampal pathway. It appears to have a negative modulating influence on cholinergic transmission, suggesting that it might interfere with memory formation.

The apparatus was a starburst maze consisting of a start box, and 5 radiating alleys: 4 level alleys and 1 baited ascending alley with a grid floor. THe subjects were 20 Sprague-Dawley rats, handled for 10 days, cannulated in the body of the lateral ventricles and deprived to 80% of initial weight. Each rat was infused i.c.v. over 6 mins. with 8µg galanin in 24µl saline or with saline alone. Twenty mins. after completion of infusion, each rat was placed in the maze and observed under "blind" conditions for number of errors (blind alleys entered) and latency to reach reward. Each rat's speed score was 100 sec./latency. One day later, each rat was retested in the maze. Each rat's retention scores were its decrease in errors and increase in speed between the single training trial and the retention trial. Galanin-infused rats showed significantly less retention by both measures. (* p<.05, ** p<.01 vs. saline).

M ± SEM	SALINE I.C.V.	GALANIN 8µg I.C.V.
ERROR DECREASE	3.1 ± 0.9	-1.1 ± 0.9 **
SPEED INCREASE	4.1 ± 1.3	0.7 ± 1.1 *

549.4

"Reproductive Memory" - A New Model for the Study of Recognition Processes in the Male Rat: Effect of Vasopressin Antagonist Administration. T. Mencio-Wszalek and V.D. Ramirez. Program in Neuroscience, University of Illinois, Urbana, Illinois, 61801.

Studies have shown that arginine vasopressin, AVP, modulates memory processing in the rat (De Wied, 1983, Prog. Brain Res., 60:155). Current work in our laboratory, using a novel model of recognition in the rat, has sought to assess the role of AVP in the rat's ability to recognize a familiar female conspecific. Sexually naive adult male rats injected intraperitoneally with either 0.5 ml of saline or 0.5 ml of the V1 antagonist, ([β-mercapto-β, β-cyclopentamethylene-proprionyl^1, O-Met-Tyr^2-Arg^8]-VP) at a concentration of 10ug/100ul, ten minutes before the trial had no effect on the animal's ability to recognize a familiar female conspecific, i.e., males rapidly mounted and directed significantly less olfactory investigation toward a familiar female conspecific (latency to mount during second interaction period = 74±11 seconds as compared with latency = 300 seconds in first interaction period). In contrast, adult male rats which received intraperitoneal injections of the V2 antagonist, ([d(CH_2)5 D-Ile^2, Ile^4]-VP), failed to recognize sexually receptive female rats with which they had interacted thirty minutes before (latency to mount = 300 seconds). Furthermore, characterization of our model has shown that recognition within reproductive interactions persists for 120 minutes and is dependent upon the consecutive introduction of the same female rat to the male. These data indicate that vasopressin may be involved in this new reproductive recognition paradigm.

549.5

CENTRAL ADMINISTRATION OF A SPECIFIC V2 VASOPRESSIN ANTAGONIST BLOCKS THE MALE RAT'S ABILITY TO RECOGNIZE A FAMILIAR CONSPECIFIC FEMALE. S.M. Sigler, T. Mencio-Wszalek, and V.D. Ramirez. Dept. of Physiology and Biophysics, Univ. of Illinois, Urbana, IL 61801.

Studies in our laboratory have demonstrated the role of arginine-vasopressin in recognition and memory processing in the rat with regard to reproductive behavior . Male Sprague-Dawley rats given an intraperitoneal injection of the V2 antagonist [d(CH_2)$_5$ D-Ile$_2$, Ile$_4$]-AVP did not show the rapid latency to mount a familiar sexually receptive female conspecific characteristic of the saline injected controls. (Mencio-Wszalek, *Abstracts, Society for Neuroscience,* 1990) Since the drug was administered systemically, possible peripheral actions responsible for this phenomenon could not be ruled out. Therefore, in order to discriminate between peripheral actions and recognition and memory processing, the drug was administered centrally via push-pull perfusion. Because the septum has been shown to be important in learning and memory, is rich in AVP receptors, and exhibits a positive feedback effect for AVP which is sensitive to V2, but not V1, antagonists (Ramirez, et al., *Journal of Neuroendocrinology,* 1990), the septum was chosen as our site of implantation and perfusion. Male rats were implanted with cannulae 7-10 days prior to perfusion. One hour prior to the first five minute interaction period between the male and female, the stylet was removed and perfusion with CSF was begun. Twenty minutes prior to the first interaction period, the V2 antagonist was administered through the push side of the push-pull cannula for 10 minutes, resulting in an infused concentration of 10ng/100µL. No change in general activity level or other behaviors was observed during any portion of the perfusion period. During the second five minute interaction period, the experimental males (n=7) showed a mean latency to mount of 260±23 sec, which was not significantly different from the 243±35 sec latency to mount exhibited 30 minutes earlier in the initial 5 minute interaction period. In contrast, the control males (n=7) showed a mean latency to mount of 35±10 sec, significantly different from the 223±24 sec exhibited in the initial interaction period. The V2 antagonist proved to be a potent inhibitor of the male's ability to recognize a familiar sexually receptive female conspecific introduced into its home cage 30 minutes after the initial 5 minute interaction period.

The actions of the V1 antagonist in the septum, as well as the V1 and V2 antagonists in other brain areas are currently under investigation.

549.6

KAPPA OPIOID AGONISTS CAN CAUSE AMNESIA FOR ONE-TRIAL PASSIVE-AVOIDANCE TRAINING IN THE TWO-DAY-OLD CHICK. P.J. Colombo, H.A. Everill*, J.L. Martinez Jr., E.L. Bennett, and M.R. Rosenzweig. Dept. of Psychology, Univ. of California, Berkeley, CA 94720

Bilateral injection of either dynorphin 1-13 amide or the kappa selective agonist U50-488 into the region of the medial hyperstriatum ventrale causes amnesia for peck-aversion training, but injection of dynorphin 1-8 does not. Chicks injected with DYN 1-13 5 min pre-training and tested either 5, 15, 30, 45, 60, 90 min, or 24 hr after training show impaired memory performance compared to saline-injected controls at 15 min, and remain impaired at all subsequent test intervals. The time-course of appearance of amnesia is consistent with impaired formation of intermediate-term memory. DYN 1-13 is most selective for kappa receptors, but also has selectivity for mu receptors. The fact that the highly kappa selective agonist U50-488 as well as dynorphin 1-13 cause amnesia in chicks, suggests that memory performance can be disrupted through kappa receptor activation.

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549.7

VASOACTIVE INTESTINAL PEPTIDE (VIP): AN AMNESTIC NEUROPEPTDE IN MICE.

J.E. Morley, J.S. Garland* and J.F. Flood. Geriatric Research, Educational and Clinical Center VA Medical Center, and Division of Geriatric Medicine, St. Louis University, St. Louis MO 63104

VIP is a neuropeptide present in high concentrations in the hippocampus. Mice were prepared for intracerebroventricular (i.c.v.) or the hippocampal injections of **VIP** or saline 24 to 48 hours prior to training on a T-maze left-right footshock avoidance task. Immediately ***after*** training saline or **VIP** was administered i.c.v. (0 to 5.0 ug) or bilaterally into the rostral portion of the hippocampus (0 to 1.0 ug total dose). When footshock avoidance training was continued one week later, **VIP** treatment resulted in dose-dependent increases in the number of trials to make 5 avoidance responses in 6 consecutive trails by either route of administration. When a **VIP** receptor antagonist ([4-Cl-D-Phe^6, Leu^{17}]-VIP) was administered into the hippocampus (0 - 2 ug), it yielded a dose-dependent improvement of retention, suggesting that **VIP** plays a physiological role in memory modulation.. The amnestic effect of **VIP** given i.c.v. was blocked by peripheral administration of memory enhancing compounds arecoline, a muscarinic agonist, and ST 587, a alpha noradrenergic agonist, but not by the gastrointestinal peptide, cholecystokinin octapeptide. Central administration of arecoline, but not NPY, blocked the amnestic effect **VIP**. The failure of NPY to block the amnestic effect of VIP was not due to using the same route of administration for both neuropeptides as centrally administered arecoline block **VIP**-induced amnesia. It is concluded that **VIP** is a potent endogenous amnestic peptide.

549.8

GP120 AND A VIP RECEPTOR ANTAGONIST IMPAIR MORRIS WATER MAZE PERFORMANCE IN RATS.

L.V. Panlilio[1], J.M. Hill[2], D.E. Brenneman[3], M. Fridkin*[4], I. Gozes[4] and J.R. Glowa[5] [1]Psychology Dept.,The American Univ., Washington,DC, 21209; [2]Peptide Design, Germantown, MD, 20874; [3]LDN, NICHD, Bethesda, MD 20892; [4] Weizmann Inst. Sci., Rehovot, Israel; [5]CNE, NIMH, Bethesda, MD 20892.

Gp120, the protein coat of HIV, has been shown to be neurotoxic to hippocampal cells in culture. These neurons support LTP, which is considered a physiological model of learning. These findings may explain, in part, the impairments in cognitive function seen in HIV positive patients. Gp120 mediated neurotoxicity in culture can be prevented by co-treatment with VIP, suggesting a basis for therapeutic intervention. In order to develop a model in which to assess this possibility, we attempted to create a comparable functional deficit in rats. We compared the effects of gp120 and a novel VIP receptor antagonist on the acquisition of Morris water maze performance. Male Sprague-Dawley rats were implanted with i.c.v. cannuli and dosed (once daily) with different agents for 7 days before exposure to the maze, and (1 hr before testing) for the entire training period. In control (saline and unoperated) rats, performance progressively improved over 15 days of training. Gp120 and the VIP antagonist impaired the acquisition of this performance. These results strongly suggest that experimental blockade of VIP receptors, or treatment with gp120, can impair performance in a learning- and memory-related task.

549.9

EFFECT OF IMMUNOACTIVE DRUGS ON MEMORY IN MICE

R.F.Ritzmann, A.Kling, A.Glasky, K.Lee*, & J.Gevorkyan*
Advanced Immunotherapeutics, VAMC/UCLA Sepulveda CA 91343

An animal model has been proposed for human memory loss which occurs during aging. This model is based on the observation that in a T-maze once a rat enters a goal box and consumes all the food, on the next trial it will enter the other goal box. By increasing the time between trial it can be determined if the rat can remember which side of the maze it entered on the previous trial. While this model has been well documented in rat it has not been tested in mouse. In the present study male Swiss Webster mice, food deprived to 80% of their free feeding weight, were tested in this model. At delays of 30 or 60 seconds a correct response occurred 75% of the time. When the delay was increased to 90 seconds the correct response rate fell to chance (46%). Since there is a considerable amount of interest in comparing immune and brain function we tested two compounds with immunomodulatory activity, AIT0082 and AIT0083, in this model. At low doses (0.5 mg/kg) both compounds improved performance at the 90 second delay to 60-65% correct. At high doses (30 mg/kg) AIT0082 improved performance to 80% correct, but AIT0083 treated mice preformed at chance level. Further studies indicated that AIT0083 at the high dose reduced performance at both the 30 and 60 second delays to chance while saline injected mice were correct 75% of the time.
Supported by VAMC Research Service & a grant from AIT.

549.10

AMYGDALA INJECTIONS OF DIAZEPAM FACILITATE LONG-TERM DECREMENTS OF THE ACOUSTIC STARTLE RESPONSE IN RATS BY REDUCING FEAR/SENSITIZATION.

B.J. Young, S.A.Rabchenuk, and R.N.Leaton. Dept. of Psychology, Dartmouth College, Hanover, NH 03755.

Borszcz, Cranney, and Leaton (1989) showed that fear conditioning in an acoustic startle habituation paradigm may mask long-term habituation through a long-term sensitization process. Physiological and behavioral manipulations that reduced fear, as indexed by freezing behavior, increased the rate of response decrements. Systemic injections of the anxiolytic drug, diazepam (Young, Helmstetter, and Leaton, 1988), facilitated long-term decrements of the acoustic startle response. The amygdala may mediate the anxiolytic effects of diazepam (Nagy, Zambo, and Decsi, 1979), and lesions of the amygdala, like systemic diazepam, facilitated long-term acoustic response decrements (Leaton and Supple, 1987). Therefore, injections of diazepam directly into the amygdala should facilitate long-term response decrements. Eight rats received bilateral intra-amygdala injections of diazepam (35 ug in 1 ul) prior to habituation training in the acoustic startle chamber, and 8 rats served as vehicle-injected controls. Ten stimuli were presented on a 60-sec interstimulus interval every other day for a total of three sessions. As predicted, diazepam injections (1) significantly enhanced, long-term decrements of the acoustic startle response, (2) did not alter initial response levels, and (3) reduced freezing in the startle chamber during the early stages of training. We conclude that the facilitatory effect of diazepam on long-term decrements of the acoustic startle response is related to its fear/sensitization reducing effects, and these effects may be mediated through the amygdala.

549.11

ANXIOLYTIC AND ANXIOGENIC DRUGS ON THE EARLY ACQUISITION OF TWO-WAY SHUTTLE AVOIDANCE IN RATS.

A. Fernández Teruel, R.M. Escorihuela*, A. Zapata*, J.F. Núñez*, F. Boix*, W. Salazar* and A. Tobeña*. Medical Psychology Unit, Dept. of Pharmacology & Psychiatry, School of Medicine. Autonomous University of Barcelona. 08193-Bellaterra, Barcelona-SPAIN

Genetic evidence (the performance of selectively bred Maudsley and Roman strains); the corticosterone response; defecation scores, as well as the results obtained with procedures reducing emotivity like postnatal or adult habituation to handling, had shown that the early acquisition of the two-way shuttle avoidance is an anxiety-mediated behavior. The purpose of the present study was to add pharmacological evidence to this view by systematically testing the action of anxiolytic and anxiogenic drugs on that task. Single forty-trials sessions with mild shocks (0.4 mA-0.6 mA) were used. In the first experiments the action of sodium pentobarbital (1.25, 2.5 and 5 mg/Kg), and three benzodiazepines (diazepam 2 and 4 mg/Kg; alprazolam 1, 1.25 and 1.5 mg/Kg and adinazolam 1, 2, 4 and 6 mg/Kg) were tested. The last two experiments were carried out to test a possible pro-anxiety action of Ro 15-4513 (2, 5 and 10 mg/Kg) and FG 7142 (5, 10 and 15 mg/Kg), two partial inverse agonists of benzodiazepine receptors, wich previus data had suggested to be anxiogenic in other animal models and in men. The results showed that the measure of early acquisition of two-way active avoidance is sensitive to detect either anxiolytic or anxiogenic effects of drugs.

549.12

DIAZEPAM IMPAIRS ACQUISITION BUT NOT RECALL OF SPATIAL INFORMATION IN THE MORRIS WATER MAZE.

R.K. McNamara & R.W. Skelton. Dept. Psychology, Univ. Victoria, P.O. BOX 1700, Victoria, British Columbia, Canada, V8W 2Y2.

Diazepam (Valium®), a frequently prescribed benzodiazepine, impairs acquisition, but not retention or recall, in humans (Lister, Neurosci. Biobehav. Rev., 9:87, 1985). Diazepam also impairs place learning in the Morris Water Maze (McNamara & Whishaw, Pyschopharmacology in press). This study sought to better define the nature of this impairment.

Three groups of rats were trained (14 days, 4 trials/day) to locate a hidden platform maintained in a constant position in a pool of cool (22±1 °C) opaque water. All groups were injected IP 20 min before training with either saline or diazepam (3 mg/kg). The diazepam and saline groups received those injections throughout training but the third group (saline-diazepam) was switched from saline to diazepam after reaching criterian performance on the seventh day of testing. Relative to the saline group, the diazepam group had longer swim paths, greater heading errors, and slower swim speeds. The saline-diazepam switched group swam slower under diazepam but showed no other deficit in maze performance. When the platform was removed from the pool, the saline and saline-diazepam groups, but not the diazepam group, showed a preference for the quadrant that previously contained the platform. None of the groups were impaired when required to swim to a black, visible platform. When the hidden platform was moved to the opposite quadrant, the diazepam and saline-diazepam group both exhibited an acquisition deficit relative to the saline group. When the platform was again removed from the pool, only the saline group preferred the new quadrant. These results demonstrate that diazepam impairs the formation of new spatial memories but not the utilization of previously stored spatial information.
(This research was supported by an NSERC operating grant awarded to RWS, and UVic. fellowship to RKM).

549.13

CHRONIC FLUMAZENIL ENHANCES LEARNING OF A SWIM-ESCAPE TASK IN RATS. M. Urbancic and T.J. Marczynski. Dept. of Pharmacology, Univ. of Illinois, College of Med., Chicago, IL. 60612.

Chronic administration of the benzodiazepine receptor antagonist, flumazenil, was previously shown to have an anxiolytic effect in the elevated plus-maze test and the punished-drinking test (Urbancic, M. et al. Pharmacol. Biochem. & Behav., 35:503-509, 1990). Since the memory-enhancing effect of flumazenil was proposed (Lal, H. et al. FASEB J., 2:2707-2711, 1988) we investigated the mnemonic effects of this drug during chronic administration. Rats pretreated with flumazenil (4 mg/kg/day in the drinking water for 2 or 3 weeks) were tested for acquisition and retention of a swim-escape response in the round water tank and in the water T-maze. Flumazenil-treated group required fewer trials to reach the acquisition criterion than controls. When the rats were tested for retention 3 days after the last trial (without further drug treatment) there was no difference between the groups, but when retested on day 5 after drug withdrawal, the control group had greater difficulty reversing the maze habit than the flumazenil-exposed rats. These findings indicate that chronic flumazenil seems to combine anxiolytic action with enhancement of learning, in contrast to benzodiazepine agonists whose anxiolytic action is accompanied with impairment of learning and memory. Supported by USAF grant 87-0364.

549.14

ANXIOGENIC CENTRAL AND PERIPHERAL BENZODIAZEPINE RECEPTOR LIGANDS EXERT DIFFERENT EFFECTS IN LEARNING AND MEMORY TESTS IN RATS. P.V. Holmes & R.C. Drugan. Schrier Research Lab., Dept. of Psychology, Brown University, Providence, RI 02912.

Previous research has demonstrated that low doses of anxiogenic central benzodiazepine receptor (CBR) ligands, the beta-carbolines, improve performance in various learning and memory tests in animals if administered prior to training. The present experiments compared the effect of a beta-carboline (FG 7142) with that of a pharmacologically distinct anxiogenic compound, a peripheral benzodiazepine receptor (PBR) ligand (RO5-4864), in two tests of learning and memory in rats. As expected, FG 7142 significantly improved performance in a passive avoidance test. RO5-4864 was without effect. In a shuttlebox escape test, RO5-4864 significantly impaired performance while FG 7142 had no effect. The effect of RO5-4864 was antagonized by the specific peripheral benzodiazepine receptor antagonist, PK-11195. These results indicate that the differential impact of CBR and PBR anxiogenic ligands on performance in aversively-motivated learning tests may be a reflection of their distinct pharmacologies. Furthermore, the anxiogenic potential of a drug does not appear to be a sufficient requirement for enhancing acquisition in aversively-motivated learning. Supported by NIMH grant #MH44034-01A1 & an Alfred P.Sloan Research Fellowship, #BR-2852 to Robert C. Drugan.

549.15

REVERSAL OF DIAZEPAM-INDUCED IMPAIRMENT IN DISCRIMINATION PERFORMANCE BY RO 15-1788. S.O. COLE, Department of Psychology, Rutgers University, Camden, NJ 08102

The effects of diazepam (DZ) alone and in combination with Ro 15-1788 on the performance of a previously-learned go-no go successive discrimination were studied in male, Sprague-Dawley rats. DZ 4 mg/kg impaired discrimination performance in five successive sessions, although animals showed some tolerance to the drug's action. The impairment in discrimination performance was due to an increase in responding during no go periods of the task (errors of commission). The benzodiazepine (BDZ) receptor antagonist Ro 15-1788 (5 and 10 mg/kg) reversed the impairment in discrimination performance and reduced the number of incorrect responses in a generally dose-dependent manner when co-administered with DZ. These findings suggest that the impairment in discrimination performance by DZ is mediated by central BDZ receptor sites. When administered alone, Ro 15-1788 10 mg/kg (but not 5 mg/kg) produced a mild impairment in discrimination performance. However, in contrast to the effects of DZ, this impairment was due to both small increases in no go period responses (errors of commission) and small decreases in go period responses (errors of omission). These findings suggest that Ro 15-1788 is not a neutral antagonist but has some intrinsic action of its own.

549.16

AN ELECTROPHYSIOLOGICAL COMPARISON OF MDL 26,479, A NOVEL TRIAZOLE, AND BETA CARBOLINES IN THE HIPPOCAMPAL SLICE: EVIDENCE FOR COGNITION ENHANCING POTENTIAL. **S.M. Sorensen, J.M. Zwolshen* and T.M. Humphreys*, Merrell Dow Research Institute, Cincinnati, OH 45215.**

Compounds which enhance memory in behavioral models have been shown to augment long term potentiation (LTP) in the hippocampus. Many of these compounds also have effects on the basal population spike amplitude. The beta-carbolines with benzodiazepine inverse agonist activity have been shown to enhance learning in animals. In these experiments we compared the effects of two beta-carbolines, DMCM and beta-CCM, with those of MDL 26,479, a novel triazole which may have cognition enhancing potential, in the hippocampus.

DMCM (5 μM) and beta-CCM (10 μM), increased the basal population spike amplitude of CA1 pyramidal neurons and the magnitude of the LTP produced by a tetanizing stimulus. At higher concentrations however, these compounds produced epileptiform activity in the hippocampal slice, consistent with their high dose convulsant properties.

MDL 26,479 (20 μM) produced similar effects on the basal population spike amplitude and on LTP suggesting that this compound will also have memory enhancing potential. Unlike the beta-carbolines however, high dose MDL 26,479 did not produce epileptiform activity, consistent with behavioral evidence that this compound does not have the high dose convulsant liability seen with many beta-carbolines.

549.17

COGNITIVE DEFICITS IN 16MO F-344 RATS AND IMPROVED PERFORMANCE WITH BMY 21502. M.D. Lindner, S.L. Moon, and V.K. Gribkoff. Bristol-Myers Squibb, CNS Biology, P.O. Box 5100, Wallingford, CT 06492-7660.

Morris Water Maze testing with a 60 minute inter-trial interval and 2 trials per day for 5 days revealed that 16mo F-344 male rats were dramatically impaired relative to 2.5 mo rats in acquiring this spatial-mapping task as measured by the swim distance to locate a submerged stationary platform. Impaired visual acuity was noted in 16mo rats by longer swim distances to a platform marked with a small visible cue than 2.5mo rats. 16mo rats were also vulnerable to hypothermia. Surprisingly, 16mo rats were already 0.5°C cooler than 2.5mo rats at baseline and their rectal temperatures dropped 0.5°C from baseline after a 60sec swim while 2.5mo rats maintained their temperatures. The possibility that these non-cognitive factors, poor visual acuity and hypothermia, might completely account for the deficits in spatial mapping performance was not supported by analysis of covariance or stepwise multiple regression. Age alone was the best predictor of spatial mapping ability which suggests that the deficit in 16mo rats may be partially due to some higher cognitive deficit. In part, the older rats' spatial mapping deficit was manifest as a delay in the appearance of a trials effect (improvement from trial one to trial two). BMY 21502, which has been shown to prolong LTP (Gribkoff, Neuropharmacology, 1990, in press) accelerated the appearance and magnitude of the trials effect in 16 mo rats.

549.18

PIRACETAM AND BMY 21502 FACILITATE PERFORMANCE OF TWO-CHOICE WIN-STAY WATER-ESCAPE IN NORMAL RATS. L.W.Means, T.R.Comer* and R.Moore*. Department of Psychology, East Carolina University, Greenville, NC 27858.

Sprague-Dawley rats given either 5 or 10 mg/Kg BMY 21502, 150 mg/Kg piracetam or methylcellulose vehicle (p.o.) daily for 38 days beginning two days before training were compared on performance of a win-stay water-escape task that required the use of trial-dependent working memory. Testing was conducted in a circular water maze that was divided into a start section and two choice sections by a T-shaped metal barrier. The task involved giving the rats pairs of trials in which the location of a submerged escape platform remained in the same choice section within a pair of trials but changed semi-randomly across pairs. Rats receiving either 5 mg/Kg BMY 21502 or piracetam made more correct choices than did rats receiving only the vehicle ($p<.05$ in each case). The facilitated performance was associated with making fewer perseverative responses that resulted in errors.

550.1

CIRCADIAN FACETS OF SENSITIVITY, RHODOPSIN & RHABDOMERE IN *DROSOPHILA*. D.-M. Chen, J. S. Christianson*, R. J. Sapp* and W. S. Stark. Div. Biol. Sci., Univ. of Missouri, Columbia, MO 65211.

Microspectrophotometry (MSP) had shown that R1-6 receptors in white-eyed *Drosophila*'s compound eye have a daily visual pigment rhythm (decreasing to 60% 4 hr after light onset, then recovering, Stark *et al., J. Neurocytol.* **17**, 499-509, 1988); the rhythm persists for 2 days in constant darkness (Stark *et al., Invest. Ophthalmol. Vis. Sci. Suppl.* **30** 291, 1989); the affect of *period* mutants was equivocal. In attempting to relate visual pigment turnover, membrane cycling and sensitivity, we followed our MSP with electron microscopy (EM), morphometry and electrophysiology (the electroretinogram, ERG). EM revealed that the cytoarchitecture of membrane cycling (autophagy and renewal) in white-eyed per^+ controls remanied constant throughout a 12 : 12 :: L : D cycle. Morphometry showed that there were no significant changes in rhabdomere cross sectional area in this diel photoperiod. However, the ERG corroborated the L:D and D:D cycles of visual pigment, with these details: (1) sensitivity varied about twice as much as visual pigment; (2) sensitivity decreases preceded visual pigment decreases by several hours; (3) UV and blue sensitivities varied in parallel in L:D but UV sensitivity decreased preferentially in D:D; (4) *per* mutants (*L* = long and *s* = short) had little influence on the timing of the ERG cycle in D:D; (5) per^s had less visual pigment and lower sensitivity. Supported by NSF grant BNS 88 11062 and NIH grant RO1 EY 07192 to WSS.

550.2

CIRCADIAN PACEMAKER CONTROL OF LOCOMOTOR ACTIVITY PHASE IN BULLA GOULDIANA. M.H. Roberts and X. Xie.* Department of Biology, Clarkson University, Potsdam NY., 13699-5805.

In order to determine whether the ocular circadian pacemakers of the marine snail Bulla gouldiana, exert phase control over the circadian rhythm in locomotor activity, we measured the phase angles for entrainment of the ocular pacemakers and the activity rhythm on four different photoperiods: L:D 15:9, 12:12, 9:15, and 4:20. We found that the phase angle for ocular entrainment was progressively advanced relative to dawn as photoperiod decreased (0.31±0.94 [29], 1.85±1.36 [10], 3.00±0.74 [22], 4.92±2.30 [17]; hrs±S.D. [n]), although the phase was fixed relative to the middle of the day. This entrained phase matches predictions based upon the two-pulse non-parametric entrainment model of Pittendrigh. In contrast, activity began near dusk on all photoperiods. On subsequent release into constant conditions, the free-running locomotor activity rhythm commenced near the time of previous activity onset. Thus, activity phase on light cycles represents the entrainment of a light sensitive pacemaker. The resulting lability in phase between ocular and behavioral rhythms in Bulla exposed to light cycles suggests that the ocular pacemakers are not the only determinants of locomotor activity phase in Bulla. We conclude that the circadian system of Bulla gouldiana consists of several pacemakers, some outside the eyes, whose internal phase relationship can be modified by altering photoperiod. Supported by NS26272 to MHR

550.3

THE ROLE OF CHLORIDE CONDUCTANCE IN THE PERIOD SHORTENING OF THE CIRCADIAN PACEMAKER IN *BULLA*
S. Michel, S.B.S. Khalsa & G.D. Block Department of Biology, Gilmer Hall, University of Virginia, Charlottesville, VA 22901

The eyes of the marine snail *Bulla* express a circadian rhythm of compound action potentials (CAPs) *in vitro*. Although hyperpolarizing and depolarizing chronic treatments both lengthen the period of the pacemaker, inhibition of Cl^- conductance has recently been found to shorten the period by up to 2.5 hr. In order to address the possible mechanism underlying this period shortening, we have examined the phase at which Cl^- conductance is important for the circadian pacemaker. Detailed analysis of the CAP rhythms in Cl^- free artificial seawater (ASW, Cl^- substituted with SO_4^-) showed a significant decrease in the length of the non-spiking part of the cycle during the late subjective night, suggesting the involvement of Cl^- conductance during this phase of the cycle. The phase response curve for 6 hour pulses of Cl^- free ASW shows small phase advances during the subjective day (CT 5-11: 38 min ±16, CT 8-14: 35 min ±15) and larger advances during the subjective night (CT 14-20: +47 min ±9, CT 17-23: 93 min ±14). The observed phase advances during the late subjective night (CT 17-23) are comparable to those previously obtained with depolarization, whereas those during the late subjective day (CT 8-14) are comparable to phase shifts to hyperpolarization. Thus the all-advance phase response curve could be a result of an alteration of the circadian rhythm in membrane potential in response to a Cl^- conductance inhibition. Cl^- current could act as a passive leak damping the circadian membrane oscillations, or, alternately, be actively driven by the pacemaker. Intracellulary recordings currently underway should help identif y the role of Cl^- conductance in setting the circadian period. *Support by DFG Mi 328/1-1 to SM, NRSA NS09621 to SBSK and NS15264 to GDB.*

550.4

THE SPIKING PHOTORECEPTORS OF THE BULLA RETINA. M.E. Geusz and G.D. Block. Dept. of Biology, University of Virginia, Charlottesville, VA 22901.

When the eye of the mollusk Bulla gouldiana is maintained in constant darkness at 15°C, two circadian rhythms occur in the frequency of impulses in the optic nerve. One rhythm, in compound action potential frequency, is believed to be generated by neurons of the basal retina (Block et al., 1984). The second, a rhythm in small impulse frequency (Geusz and Page, 1990), could be generated by spiking photoreceptors of the distal retina. Impulses recorded intracellularly from the spiking photoreceptors occur in synchrony with small spikes in the optic nerve, whether they are generated spontaneously, through current injection, or by light. Impulses from the spiking photoreceptors cease briefly following a light onset, and then resume firing at an increased rate. The spiking photoreceptors display characteristics of the previously described H-type cells of Bulla, and may account for the firing pattern of small impulses in the optic nerve in darkness. Supported by NS08806 to MEG and NS15264 to GDB.

550.5

CHARACTERIZATION OF A PUTATIVE CIRCADIAN OSCILLATOR PROTEIN IN THE EYE OF *Aplysia*. U. Raju, M. Nunez-Regueiro*, R. Cook*#, and A. Eskin. Dept. of Biochem. and Biophys. Sci., Univ. of Houston, #Baylor College of Medicine, Houston, TX. 77204.

Elucidation of the circadian oscillating mechanism entails identifying its components, determining how the components interact with one another and testing whether these interactions account for properties of the circadian rhythm. A reasonable hypothesis is that proteins are components of entrainment pathways and they also are components of the oscillator. Light and serotonin (5-HT) both regulate the circadian rhythm in the eye of *Aplysia*. Therefore, we have looked for proteins whose synthesis is modified by light or 5-HT. Using 2-D gel electrophoresis to separate proteins, we found that exposure of eyes to light or 5-HT altered the incorporation of amino acids into a number of proteins. A particularly interesting finding was that a few proteins were modified by both light and 5-HT. As a result of their responses to light and 5-HT, a number of proteins may be considered putative components of the oscillator.

As a first step towards determining the role of these proteins in the circadian mechanism, we are obtaining amino acid sequences of the proteins. Protein spots were cut from 2-D gels, digested with V8 protease, and then separated on a 1-D gel. Peptides were electroblotted from the gel to Immobilon-P membrane and then placed into a gas-phase sequencer. A 38 amino acid sequence of a peptide was obtained from a protein (≈40k, pI 5.6) that was affected in opposite ways by light and 5-HT. Most exciting and important was the discovery of a significant homology (>60%) between our sequence and published sequences of a family of proteins called lipocortins. This family of calcium and phospholipid binding proteins is believed to play important roles in cellular regulation. The tentative identification of the 40k as a lipocortin has given us new ideas about where to look for the circadian oscillating mechanism. More specific hypotheses can now be tested based on the possible cellular functions of this protein.

550.6

MONOCHROMATIC LIGHT MODIFIES PERIOD LENGTH IN CRAYFISH ELECTRORETINOGRAPHIC CIRCADIAN RHYTHM. V. Inclán-Rubio and J.L. Herrera-León*. Departamento de Fisiología, Facultad de Medicina, U.N.A.M., Apdo. Postal 70-250, C.P. 04510, México, D.F.

One widespread effect of constant white light applied on electroretinographic (ERG) circadian rhythm in crayfish is changing in the period (Δt) of the freerunning rhythm. In previous works, we showed that it is possible to evoke a phase shift on this rhythm when it is exposed to a 50 min. and 400 lux light pulse with a different wavelength (λ). This work was designed in order to analyse a possible change on angular velocity ERG when monochromatic light is applied in continuous way. Adult and intact crayfish Procambarus bouvieri were monochromatic and white light adapted (λ: 465, 565 and 630 nm) with a 100 lux intensity during six days. The ERG was recorded by means of a metal electrode into the cornea to lead the photoreceptor's electrical response. The change in period (τ) length depends on the λ employed, thus, the lag between the ERG acrophase obtained with white light and with blue light was 210°, with green light (565 nm) was 20°, and with red light (630 nm) was 180°. These results indicated that the net change in angular velocity causes the observed change which depends on one photoreceptor's group altered by the λ used.

550.7

EFFECT OF TEMPERATURE UPON ULTRADIAN AND CIRCADIAN ERG RHYTHMS IN THE CRAYFISH. M.L. Fanjul-Moles, J.A. Prieto-Sagredo* and B. Fuentes-Pardo. Depto. de Fisiología, Fac. de Medicina y Depto. de Biología, Fac. de Ciencias, U.N.A.M. Apdo. Pos. 70-371, México D.F. México.

The Electroretinogram (ERG) in the crayfish shows during the earlier stages of ontogeny a clear ultradian rhythm (UR) ($\tau \simeq 4$ hs) which anteceds the circadian rhythm (CR). On the other hand, a persistent ERG amplitude CR with superimposed ultradian oscillation has been established in the isolated eyestalk of the adult crayfish. These findings suggest that the UR represent the output of a multioscillator system which, when in phase, gives rise to the overt CR. The aim of this work was to analize the mechanism of temperature compensation in both the juvenil stages and the excised eyestalk of the adult in order to prove that the UR and CR share this property. ERG recordings were performed individually in both preparations under darkness for at least six days at high and low temperatures; the oscillation period was measured and Q10 calculated. In the juvenil animal Q10 was 1.01 for the UR and 1.05 for the CR. For the eyestalk the Q10 values was 1.19 and 1.52 for UR and CR respectively. These results show that: a) UR shares with the CR the ability to compensate temperature; b) This mechanism is present since very early stages of development and persists in the adult animal. c) The isolated eyestalk maintains the temperature compensation mechanism but to a minor degree.

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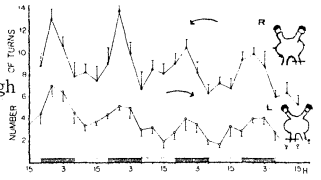
550.8

IS LEFT DOMINANCE INVOLVED IN THE COMMAND OF CIRCADIAN LOCOMOTION IN CRAYFISH? Elvira Barrera-Calva* and B. Barrera-Mera. Depto. de Fisiología, Facultad de Medicina, UNAM Apdo. Postal No. 70-250, 04510 México, D.F. México.

The protocerebrum of crayfish contains a pair of bilaterally located circadian pacemakers driving retinal sensitivity (B. Barrera-Mera, et. al., Soc. Neurosci. Abstr. 5, 1979) and glucose concentration in hemolymph (B. Barrera-Mera and S. March-Mifsut, Soc. Neurosci. Abstr. 11, 1985). In order to learn if hierarchical control exists between such cephalic pacemaking structures, we measured crayfish locomotion before and after unilateral (Left or Right) transection of either circummesophageal commisure. Both kinds of preparations resulted in a different amount and sense of movement throughout the day (Fig. 1), even though the recorded number of turns were obtained in constant conditions of light and darkness. We propose that the driving role for locomotion is continually exerted in a preferential manner from the left protocerebral circadian pacemakers.



Circadian variations in the amount of circling behavior of crayfish with surgical transection of either circumesophageal commisure (R L). Measurements were obtained each four hours

550.9

[14C] LEUCINE UPTAKE IN BRAIN DURING THE HIBERNATION CYCLE. T. S. Kilduff and H. C. Heller*. Depts. of Psychiatry and Biol. Sci., Stanford Univ., Stanford, CA.

Changes in arousal state such as sleep and hibernation are accompanied by changes in synthesis of neurotransmitters and neuropeptides. As an index of protein synthesis, 1-[^{14}C]-leucine uptake was measured autoradiographically in 50 brain regions of the squirrel Citellus lateralis during various stages of hibernation. Squirrels were injected with 50uCi of 1-[^{14}C]-leucine through chronic jugular catheters during euthermia (T_b=37^{o}C), entrance to hibernation, deep hibernation and arousal. The label was allowed to incubate for 2hr in euthermia and up to 2d in hibernation. Incubations were terminated by pentobarbital overdose, the brain removed, sectioned in a cryostat and exposed to X-ray film. The greatest levels of 1-[^{14}C]-leucine uptake are observed during euthermia in the hippocampal pyramidal cell layer, pontine nuclei, and habenula and the lowest levels observed in white matter. The pontine nuclei and the substantia nigra pars compacta show much greater levels of 1-[^{14}C]-leucine uptake in the euthermic squirrel than reported previously for regional protein synthesis in the rat brain. During hibernation, the fasciculus retroflexus is heavily labelled whereas very little labelling is evident in this region during euthermia. Tissues have now been formalin-fixed to determine actual 1-[^{14}C]-leucine incorporation into protein in different nuclei (Supported in part by the Upjohn Co.).

550.10

WARMING UP TO SLEEP?: HIBERNATING GROUND SQUIRRELS SPEND EUTHERMIC SPONTANEOUS AROUSALS IN REM AND NONREM SLEEP. B.M. Barnes, A. Strijkstra* and S. Daan*, Institute of Arctic Biology, University of Alaska, Fairbanks, AK 99775 and Zoological Laboratory, University of Groningen, PO Box 14, 9750 AA Haren, The Netherlands.

Hibernation in mammals consists of long intervals of deep hypothermia or torpor (1-3 weeks at body temperatures circa 0^{o}C) that are interspersed by short arousals to euthermy (8-20 hrs at 37^{o}C). These spontaneous arousals are very energetically expensive compared to torpor, and their functional significance remains unknown. This study sought to test the hypothesis that hibernators enter these arousals in order to sleep. Sleep may be a temperature dependent process that cannot occur during hypothermia; however, the need for sleep may continue to accumulate during torpor, albeit at a reduced rate. Spontaneous arousals to high body temperatures may be permissive of the required sleep. Arctic and golden-mantled ground squirrels (*Spermophilus parryii and S. lateralis*) were implanted with cranial and subcutaneous electrodes for measurements of EEG and EMG and kept from Dec-Apr in continuous darkness and 2^{o}C. Torpor patterns were followed by recording skin temperatures. Paper traces of EEG and EMG and EEG signal-power density in frequency bands from 0.5-13.5 Hz were recorded for spontaneous and induced arousals following different intervals of torpor. Traces were scored for wakefulness, nonREM (slow-wave) and REM sleep; power density is a measure of sleep intensity (Daan et al. Am J Physiol 246:R161). Ground squirrels spent >90% of their 14-20 hr arousal periods in sleep, directly entering sleep upon rewarming and then alternating between nonREM and REM sleep. Power density decreased during arousal sleep, and, in most animals, total power was less after short vs. long torpor bouts. A functional relationship between sleep and periodic rewarming from hibernation counters ideas of torpor as an extension of sleep and of sleep having evolved as a energy saving adaptation.

550.11

THE PERIOD OF THE CIRCADIAN OSCILLATOR PERSISTS IN SPACE WITH A DECREASED FREQUENCY. J.S. Ferraro, F.M. Sulzman*, D.J. Happe-Shelton*, M.L. Pence*, S.R. Golay*, K.H. Ekborg* and J.A. Dorsett*. Physiology Dept, Southern IL Univ, Sch of Medicine, Carbondale, IL 62901.

An experiment, designed to examine the endogenous nature and physiology of the circadian oscillator under microgravity conditions, was conducted aboard shuttle flight STS-32 in January of 1990. The circadian rhythm of conidiation (asexual spore formation) in *Neurospora crassa* was monitored in "race" tubes containing an acetate medium with agar, Vogel salts, casamino acids and 3% Brij (50% inoculated with BND and 50% with BND/CSP strains). The tubes, inoculated at one end, were grown in constant light for two days. Growth fronts were marked and the tubes were then place into constant dark. They were integrated into one of three sections of the light-tight mid-deck locker, which was delivered to the orbiter and fixed to the mid-deck wall. Nine hours after launch, mission specialists marked the growth fronts of tubes in the "Blue" and "Red" sections. "Red" contained 15 race tubes covered by a red filter 99.9% dense for wavelengths >610nm. "Blue" contained 25 unfiltered race tubes. The growth-front marking also occurred to all sections, including "White" (10 unfiltered tubes), at approximately launch plus 5 days and 22 hours (L+5D/22h), L+8D/22h and L+10D/3h. The conidiation rhythm persisted with a robust amplitude in-flight. In fact, the amplitude was more robust than that seen on a preliminary experiment flown in 1983 on STS-9. The average period of the oscillation was increased by as much as four hours, over the eleven day flight. These results suggest that circadian oscillations are endogenously generated by an internal clock; however, gravity and/or the absence of gravity, like light and/or the absence of light, can affect the period of the rhythm. Supported by NASA grants NAG 2-361 and NAG 2-452 (JSF).

550.12

CIRCADIAN CLOCK REGULATES ROD-CONE SHIFT IN THE RETINA OF THE JAPANESE QUAIL. H. Uchiyama, N.F. Buelow and R.B. Barlow,Jr. Inst. Sensory Res., Syracuse Univ., NY 13244-5290

Animals with duplex retinae can shift from rod- to cone-mediated vision. Associated with this shift is a change in maximum spectral sensitivity known as the Purkinje shift. We studied the Purkinje shift in the Japanese quail, a galliform bird with duplex retina, and found both ambient illumination and a circadian clock control the Purkinje shift in this bird.

Electroretinograms (ERG) were recorded intraviterally from immobilized, urethane-anesthetized animals in response to flashes of monochromatic light. Spectral sensitivities were measured (b-wave, constant response criterion) under different lighting conditions from birds previously maintained on a 12:12 LD cycle. Dark-adapted animals exhibited an endogenous rhythmic Purkinje shift. Their maximum spectral sensitivity was about 500 nm during subjective night and 600-625 nm during subjective day. Light-adapted animals had maximum spectral sensitivity of about 600 nm regardless of time of day. For animals maintained in constant darkness up to 35 hrs, the rhythmic changes in sensitivity had a period of about 24 hrs and were greatest for blue light stimuli (425-475 nm).

We conclude that a circadian clock modulates the functional organization of the Japanese quail retina. It shifts the retina from rod dominated to cone dominated with time of day. Others have reported that intraocular oscillators modulate retinal levels of melatonin, dopamine and related substances in galliform birds. The same oscillators may have a role in the circadian rod-cone shift in birds.

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550.13

CIRCADIAN CONTROL OF *MANDUCA SEXTA* FLIGHT. H.K. Lehman. ARL-Division of Neurobiology, University of Arizona, Tucson, AZ 85721.

Circadian clocks have been described in a wide variety of organisms and are known to control several physiological processes, yet the neurotransmitters that mediate these processes are less well known. I have focused on the circadian flight of an insect, *Manduca sexta*, to study the transmitters and pathways in which a circadian clock controls behavior.

Flight activity was recorded from dorsal longitudinal flight muscles of individually restrained moths. Flight activity persisted in constant darkness, had a period length slightly less than 24 hours, and could be phase shifted. Circadian flight activity was abolished by severing the connectives between the suboesophageal and thoracic ganglia or removal of the brain, suggesting that the brain is the site of the circadian clock. Octopamine titers were then measured in the brain and hemolymph over a 24 hour period. Concentrations in the brain ranged from 2.5 to 8.6 to pmoles/brain and in the hemolymph from < .10 to .85 pmoles/50 µl. The greatest concentrations in each tissue were detected between 10 PM and 4 AM, coincident with peak levels of flight activity. In addition, injections of the octopamine agonist, chlordimeform, produced long lasting flight activity. Recently, immunocytochemical techniques have been used to visualize octopaminergic neurons in the brain, and studies are underway to characterize these neurons in order to determine which may participate in the circadian control of flight.

Supported by grants to J. G. Hildebrand.

550.14

POSTNATAL HANDLING REDUCES THE AMPLITUDE OF THE DAILY TEMPERATURE RHYTHM IN THE RAT. S. Amir and A. Schiavetto*. Center for Studies in Behavioral Neurobiology, Concordia Univ., Montreal, Que., Canada.

Adult male Sprague-Dawley rats (90 days old) subjected to brief daily periods (60 min) of handling and separation from the mother during the first three weeks of life (H) exhibited significant reductions in the amplitude of the circadian temperature rhythm compared to non-handled controls (NH). The changes in rhythm amplitude persisted across different environmental lighting conditions or ambient temperatures. The amplitude of the daily temperature rhythm (mean±s.e.m., in °C) in H rats (n=5) tested for 10 days under 12h:12h light-dark cycle and ambient temperature of 24°C was 0.32 ± 0.01 compared to 0.46±0.01 in NH (n=5; $p<0.01$). When tested under similar lighting conditions but at ambient temperature of 4°C the amplitude values were 0.38±0.03 and 0.58±0.02 for H and NH rats, respectively ($p<0.01$). When tested for 10 days under constant light and normal ambient temperature the amplitude of the temperature rhythm was 0.38±0.03 and 0.52±0.03 for H and NH rats ($p<0.01$). Under constant light and ambient temperature of 4°C the amplitude values were 0.36±0.02 and 0.56±0.03 for H and NH rats ($p<0.01$). Previous studies have shown that brief periods of daily handling and separation of newborn rats from the mother result in changes in neuroendocrine and behavioral responses to stress which persist into adulthood. The present results show that such treatment also has a long-lasting effect on the amplitude of the circadian temperature rhythm.

550.15

STRAIN-DEPENDENT EFFECTS OF INCREASING LIGHT INTENSITY ON THE CIRCADIAN RHYTHMS OF LOCOMOTOR ACTIVITY IN MICE. A.R.Mayeda*, J.R.Hofstetter*, and J.I.Nurnberger,Jr. Institute of Psychiatric Res., Indiana Univ.Med.Sch., Indianapolis, IN 46223.

To investigate the genetic determinants of the effects of light on the circadian rhythms in mammals, male mice of several inbred strains were monitored under constant light of successively increasing intensity. They were housed individually, and their locomotor activity was monitored over the course of several months using photoelectric beams. The C57BL/6J mice seemed to have an all-or-nothing response to light. Their period ( ) increased with a change from constant dark to 8 lux constant light but did not increase further when the light intensity was increased to 93 lux. Furthermore, the daily bimodal pattern of locomotor activity of the C57 mice was lost when going from light:dark or dark:dark conditions to constant light. In contrast the period of activity for the DBA/2J mice increased as the light intensity increased from 0 to 8 lux and increased again as the light intensity went to 93 lux.

550.16

CIRCADIAN RHYTHMICITY IN SHR AND WKY RATS: EFFECTS OF LIGHT INTENSITY. A.M. Rosenwasser, Dept. of Psychol., Univ. of Maine, Orono, ME 04469.

Recent studies from this laboratory suggest that a noradrenergic mechanism may interact with light intensity to alter free-running circadian period. In the course of these studies, it was noted that the spontaneously hypertensive (SHR) and Wistar-Kyoto (WKY) rat strains, known to display characteristic behavioral and neurochemical differences, also differed in free-running period under constant light. In the present study, circadian drinking rhythms of SHR and WKY rats were monitored under a series of increasing light intensities, beginning with constant darkness. Least-squares spectral analysis was used to quantify parameters of free-running rhythms. As expected, periods lengthened, amplitudes decreased, and spectral profiles became more complex, with increasing light intensity. Strain differences in period, characterized by longer periods in the WKY strain, were seen only under the highest intensities, while strain differences in amplitude, characterized by greater amplitudes in the WKY strain, were seen only under the lowest intensities. In contrast, the two strains showed similar spectral profiles, and appeared equally likely to show disrupted rhythmicity under bright light. The strain by light intensity interactions seen in this study may be due to strain differences in noradrenergic systems.

550.17

RESPONSES OF MONTANE VOLES TO INCREMENTAL PHOTOPERIODS. C.N.Rowsemitt and P.J.Berger*. Dept. of Biology, Univ. of Utah, Salt Lake City, UT 84112.

Previous work suggests that montane voles may respond to small changes in day length at the winter solstice (WS) to schedule changes in body weight (Petterborg, L.J. Can.J. Zool. 56:431, 1978). We housed adult male voles (raised under LD 16:8) under decreasing photoperiod in the laboratory (-2 min/day) from Oct. 18 until WS at which time animals were divided into 3 groups: A-natural mimic (+2 min/day), B-slope hold (-2 min/day), and C-solstice hold (LD 9:54:14:06). Controls were housed under LD 12:12 (group D) or LD 16:8 (group E) after Oct. 18. Group F was housed in an unheated shed with natural lighting. Animals were sacrificed on Feb. 21. Groups A, C,D, and F lost weight between mid-Nov. and WS and maintained stable weights for the following month. Group B lost weight between mid-Nov. and WS but gained weight in the following month. Group E gained weight in both of these intervals. Analysis of covariance of log testes weight with log body weight as covariate demonstrates a significant effect of treatment. Group A has higher reproductive function than group B, with a trend existing when groups A and C are compared. Thus, post-solstice changes in growth rates appear to be spontaneous. However, gonadal function may respond to incremental changes in photoperiod.

550.18

THE PERIOD OF THE HAMSTER CIRCADIAN PACEMAKER SHORTENS IN CONSTANT LIGHT OF INTERMEDIATE LEVELS. Dwight E. Nelson and Joseph S. Takahashi Department of Neurobiology and Physiology, Northwestern University, Evanston, Illinois 60208.

The visual sensitivity of the hamster circadian pacemaker to brief stimuli has been studied extensively. Using the phase-shifting response to brief light pulses as an assay we have shown that this pacemaker can integrate the total photons in a single stimulus for 300 s and does not light-adapt to ambient illumination. We have measured the photic sensitivity of the freerunning period of the circadian activity rhythm to *continuous* irradiance levels that span 6 log units. Golden hamsters were maintained in constant light (530nm; HW=45nm) of 10^8 to 10^{14} photons cm^{-2} s^{-1} and the freerunning period of activity was measured after 1, 6 and 9 weeks. One group remained in darkness as a control. During week 1 of constant conditions the period of the activity rhythm shortened monotonically with increasing irradiance. At light levels less than 10^{12} photons cm^{-2} s^{-1} the freerunning period was not different from the period measured in darkness (23.99 ±0.01 h; mean ± SEM). At higher irradiance levels the period was shorter (23.70 ±0.03 h at 6 x 10^{12} photons cm^{-2} s^{-1}). After 6 weeks in constant conditions a biphasic relationship had developed between the constant irradiance and the freerunning period. The freerunning periods of hamsters in low and high levels of illumination were similar (approximately 24 h) yet the periods of animals kept in intermediate levels of illumination (approximately 10^{11} photons cm^{-2} s^{-1}) were significantly shorter (23.80 ± 0.06 h). After 9 weeks in constant conditions the period of the rhythm for hamsters in intermediate levels of constant light remained significantly shorter (23.80 ±0.02 h) than the periods for both animals in darkness (24.04 ±0.02 h) and the highest levels of illumination (24.20 ±0.11 h). The *shortening* of the freerunning period in hamsters maintained in intermediate levels (≈ 10^{11} photons cm^{-2} s^{-1}) of constant light has never been quantified however it has been predicted by Daan and Pittendrigh (*J.Comp.Physiol.* 106:267,1976) based upon the phase-shift responses of the hamster to brief light pulses. Our results suggest that changes in the freerunning period of the hamster circadian pacemaker to light can be predicted by the sensitivity of the pacemaker to brief light pulses.

550.19

EFFECT OF NOVELTY ON OVERT AND CENTRAL COMPONENTS OF ORIENTING AND ALERTING. L.D.Sanford*, W.A. Ball*, R.J.Ross, P.J.Gresch* and A.R.Morrison. Depts. Anim. Biol. and Psychiatry, Univ. of Penn. and VAMC., Phila, PA 19104.

Spontaneous ponto-geniculo-occipital (PGO) waves in REM, elicited PGO waves (PGO_E), acoustic startle (ASR) and orienting responses (OR) may reflect the operation of related components of alerting. To determine if mechanisms underlying PGO_E are central correlates of OR or ASR, we presented novel stimuli (NS) in waking and measured behavioral responses and PGO_E. Four cats received 140 consecutive stimuli (90 ms, 100 dB, 2 s ISI): 80 pure tones (T; 1 kHz), 20 white noise bursts (WN; 20 Hz - 50 kHz) plus 40 T. A week later the order of T and WN was reversed. The repeated initial presentation of T or WN alone resulted in a linear decrease in OR with no significant change in PGO_E magnitude (MAG). ASR was inconsistent yet did occur on the first T or WN trial in 3 cats. WN presented after T resulted in increased OR with responses qualitatively similar to those observed initially. PGO_E showed a significant interaction across days with the change from T to WN resulting in increased PGO_E MAG and the change from WN to T producing no increase. Novelty did not affect ASR. Thus, OR showed rapid habituation that was reversed by NS; PGO_E MAG did not readily habituate yet did increase in response to NS. This suggests that experience may dissociate overt and central mechanisms of orienting and alerting, but exposure to NS may quickly reintegrate OR and PGO_E without enhancing ASR. Supported by MH-42903, MH-18825, and the D.V.A. Med. Res. Serv.

550.20

HIGHLY SELECTIVE REM-SLEEP DEPRIVATION IN RATS SUPPRESSES NON-REM-SLEEP DELTA POWER. J. H. Benington and H. C. Heller*, Dept. of Biological Sciences, Stanford University, Stanford, CA 94305.

Traditional approaches to REM-sleep deprivation (RD) in animals produce non-specific stress and early loss of non-REM sleep (NREMS) time. We have developed a highly selective method of RD, using mild electrical stimulation (4-6Hz, 15-30uA) of the dorsal raphe nucleus. RD by this method produces no loss of NREMS time, even in the first hour. Stimulated arousals are indistinguishable from normal arousals from REMS and usually last just 10-20 seconds.

EEG power spectra for 1-2 hour RD during the rest period were compared to time-matched baseline recordings and to control recordings in which NREMS was fragmented at circa 100 second intervals without disrupting REMS. RD produced a 35.8% suppression of EEG delta (0.5-4.0Hz) power in NREMS, as compared to 15.7% for NREMS fragmentation ($p<0.01$, two-tailed t-test).

The suppression of NREMS delta power by RD represents a deprivation of deep NREMS (slow-wave sleep) in spite of no loss of NREMS time, suggesting that cognitive and behavioral deficits following RD may be partly attributable to diminished NREMS quality.

Supported by a grant from the Upjohn Company.

550.21

FOOD DEPRIVATION AND RESTORATION AT DAWN DISTURBS THE CIRCADIAN FEEDING RHYTHM IN RATS. J.S. Kruse, K. Krauchi* and A. Wirz-Justice*.
Psychiatric University Clinic, Basel, Switzerland CH-4025.

In analogy to Borbely and Daan's two-process model of sleep, we studied feeding regulation in rats. We measured food intake after deprivation at different circadian phases and under different lighting conditions, to separate circadian from homeostatic effects.

Circadian rhythms of food intake were continuously registered with a computerised food hopper system in 10 male Wistar/Fullinsdorf rats (400-500 g) before and after 1-3 days of food deprivation that began either at the onset or offset of subjective day. In order to reduce masking effects of light, experiments were performed under skeleton 12:12 photoperiods with 1 hour light pulses, as well as under constant DD or RR conditions.

Two days of food deprivation followed by restoration at dawn increased the daily mean meal duration and meal size, without changing circadian amplitude. Meal number and intermeal interval were reduced in circadian amplitude without changes in daily mean values. In contrast, no marked rebound of any variable was found after food restoration at dusk. Thus, food restoration at the circadian antiphase induced a rhythm disturbance of increased feeding in the subjective day. This was seen under all lighting conditions, lasted about 3 days, and disappeared gradually. Additionally, under a skeleton photoperiod, food restoration at dawn, but not dusk, induced an advance (1 hour) in the time of cessation of eating, reducing the duration of the activity phase of feeding.

550.22

EFFECTS OF RESTRICTED FEEDING SCHEDULES USING A PALATABLE DIET ON CIRCADIAN ACTIVITY RHYTHMS IN HAMSTERS. H. Abe and B. Rusak. Dept. of Psychology, Dalhousie Univ., Halifax, Nova Scotia, Canada B3H 4J1.

The effects of daily feeding schedules have not often been studied in golden hamsters because they do not readily adapt to temporally restricted food access. We used a modified restricted feeding schedule with a highly prefered palatable diet and moderate food deprivation to examine whether feeding schedules influence the hamster circadian system. Hamsters were kept in constant dim red lighting and given sufficient food (an applesauce/chow combination) at a fixed time of day to maintain 80-90% of free-feeding weights. When body weights were reduced, many hamsters showed clear anticipatory increases in activity before the daily feeding times. Some hamsters also showed entrainment of their freerunning circadian rhythms to these schedules. When the feeding time was advanced by 4.5 hrs, the rhythms shifted toward the new feeding time and entrainment was re-established in some animals. No entrainment and no anticipatory activity were observed in control animals given excess palatable diet at a fixed time. These results suggest that in an appropriate motivational state, hamsters can be entrained by non-photic cues acting directly or indirectly on the circadian pacemaker. We are now examining the effects of suprachiasmatic nucleus (SCN) lesions on anticipatory activity and food-entrained rhythms to see whether hamsters have a food-entrainable system outside the SCN.

Supported by the Japan Society for the Promotion of Science and grants from the NSERC and MRC of Canada.

EPILEPSY: ANIMAL MODELS

551.1

MAPPING WHOLE BRAIN ACTIVITY DURING DRUG INDUCED SEIZURES WITH VOLTAGE SENSITIVE DYE. D.S. SACKS and R.M. DASHEIFF. Dept. of Veteran Affairs, VAMC, University Dr., Pgh, Pa, 15240 and Univ. of Pittsburgh Epilepsy Center.

The functional interrelationship between electrical activity and brain function is a central concern in the study of epilepsy. In vivo mapping of neural pathways during seizures can uncover new structure-function relationships. This study used the voltage sensitive dye diO-C(2)-5 as a fluorescent marker of electrical activity in neural structures during drug induced seizures in awake animals. The technique allows a complete histological survey of the brain using computerized image analysis (Exp. Neurol 105:189-196, 1989). The rats (yoked from surgery to image analysis) received one of four treatments prior to dye injection: 1) intrajugular injection of 0.25 mg/kg of bicuculline (BIC) + DMSO vehicle, 2) intrajugular injection of DMSO alone, 3) i.p. injection of 12 mg/kg Kainic Acid (KA) + saline vehicle, or 4) i.p. injection of saline alone. EEG activity was recorded via hippocampal electrodes and the dye injection timed to coincide with seizure activity. The neural activity of the septum, amygdala, anterior thalamus, hippocampus, mammilary bodies, substantia nigra, and entorhinal, frontal and posterior cortices was mapped.

Past work had shown that different patterns of hippocampal hyperpolarization and depolarization occurred during seizures induced by KA and BIC. We hypothesized that both a spatial and temporal pattern of polarization throughout the brain would be characteristic of different seizure inducing agents. These patterns should provide insight into both mechanisms of seizure propagation and strategies for medical interventions.

551.2

ALPHA-2 ADRENERGIC CONTROL OF THALAMIC OSCILLATION. G. Buzsáki, C. Slamka*, A. Salami*, F. H. Gage, Z. Horváth* and M. Hsu. Dept. of Neurosciences, UCSD, La Jolla, CA 92093

The effects of alpha-adrenergic drugs on neocortical high voltage spindles (HVS), reflecting thalamic oscillation, was investigated in freely moving rats. Bilateral microinjections of the alpha-2 agonists, xylazine and clonidine into the n. ventralis lateralis area of the thalamus, but not into the hippocampus or corpus callosum, as powerfully increased the incidence and duration of HVS as when these drugs were given peripherally. The HVS-promoting effect of clonidine was antagonized by prior intrathalamic injection of the alpha-2 antagonist, yohimbine. The amplitude of the HVS was increased by picomole amounts of unilaterally-injected clonidine. Neurotoxic destruction of the thalamopetal noradrenergic afferents by intracisternal or intrathalamic injection of 6-OHDA increased the incidence of HVS. Importantly, intrathalamic administration of xylazine continued to induce HVS after destroying the thalamic noradrenergic terminals. Downregulation of alpha-2 receptors by amitryptilene reduced the effectiveness of xylazine. We suggest that a major action of alpha-2 adrenergic drugs on thalamic oscillation is mediated by postsynaptic alpha-2 adrenoceptors located on the thalamocortical neurons and that the final physiological action of norepinephrine on thalamic oscillation is a function of the relative density and affinity of alpha-1 and alpha-2 adrenoceptor subtypes.

551.3

CHRONIC AND ACUTE EFFECTS OF TETANUS TOXIN IN RAT NEOCORTEX. Y. Chagnac-Amitai, K. Brenner* and M.J. Gutnick, Dept. of Physiology, Ben-Gurion University of the Negev, Beersheva, Israel.

We previously showed that injection of 50 MLDS of tetanus toxin (TT) into rat neocortex produces a potent chronic epileptic focus (J. Physiol. 413:34P, 1989). We now describe the neuronal activities in neocortical slices prepared from both hemispheres of rats that had been unilaterally injected with a minute quantity of TT (2-10 MLD in 0.5 μl) 16 hours to 35 weeks earlier. All slices showed hypersynchronous activity; spontaneous discharges were observed in 50% of the experiments. These took the form of prolonged, negative field potentials which coincided with intracellular DSs. In most slices, there was evidence of residual postsynaptic inhibition despite the profound epileptogenesis. However, IPSPs were only recorded in restricted regions of a given slice. APV (20 μM) abolished most of the DS, often unmasking a late, prolonged hyperpolarization. This is in contrast with the moderate effect of NMDA blockers on focal epileptogenesis induced by acute convulsants.

Hypersynchrony also followed exposure of normal slices to TT. This effect was dose and time dependent. Although fast IPSPs were selectively reduced at first, prolonged incubation led to eventual blockade of all synaptic transmission.

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551.4

THE ROLE OF THE SPINAL CORD IN EXPERIMENTAL EPILEPSY. K.S. Blisard, K. Fagin*, O.U. Scremin. Univ. of Cincinnati Med. Ctr., Cincinnati, OH; Univ. of New Mexico Med. Ctr., Albuquerque,NM; Veterans' Admin. Med. Ctr., Albuquerque, NM

Although most seizures are thought to be mediated by higher structures, under certain circumstances the spinal cord can play an important role. We used frogs with thoracic spinal cord transections to investigate this question for several chemical epileptogens. Strychnine induced status epilepticus with tonic-clonic convulsions; these seizures were identical in the intact or cord-transected animal. This is consistent with the seizures originating in the spinal cord. Cis-platin (cis-diamminedichloro-platinum II) caused tonic-clonic seizures in intact frogs; after cord transection, seizure activity occurred in the upper extremity only while the lower extremity remained flaccid. These observations suggest that the seizures originated in structures above the level of the cord. Kainic acid and pentylene tetrazole gave intermediate results. With these agents, cord transected frogs showed some activity of the lower extremities (but decreased from that seen in the upper extremities), suggesting that spinal cord activity may contribute to seizures.

551.5

ROLE OF MK-801 IN THE γ-HYDROXYBUTYRIC ACID (GHB) MODEL OF GENERALIZED ABSENCE SEIZURES IN RATS. P.K. Banerjee and O. Carter Snead III Div. of Neurology, Childrens Hospital Los Angeles, Dept Neurology, Univ. South. Calif. Sch. Med., Los Angeles, Ca 90027

γ-Hydroxybutyric acid (GHB), a naturally occurring metabolite of GABA, induces bilaterally synchronous spike wave discharges (SWD) associated with behavioral changes reminiscent of petit mal seizures when given to animals. The GHB-treated animal thus represents a useful experimental model of generalized absence seizures. Although there is a wealth of data concerning excitatory amino acid (EAA) mechanisms in experimental generalized convulsive seizures, there is little information concerning EAA in animal models of absence seizures. In the present study, the effect of the noncompetitive antagonist of the NMDA receptor, MK-801, on GHB-induced SWD was ascertained using dose response and time course studies in rats chronically implanted with cortical electrodes which allowed continuous EEG recording in the freely moving state during all experiments. The GHB model of experimental absence seizures was standardized and quantitated as previously described (Snead, Epilepsia 29:361, 1988). The dose range of MK-801 used in these studies was 0.1-1 mg/kg.

MK-801 had a complex biphasic effect on the GHB-induced seizure in that it produced significant prolongation of the SWD in the early stages of seizure (25% increase), but attenuated later stages of the absence-like episodes. Lower doses (0.1 mg/kg) of MK-801 resulted in a 35% delay in latency to onset of SWD while doses of MK-801 in excess of 1 mg/kg resulted in trains of spike activity when given alone.

These data raise the possibility that EAA-mediated mechanisms may be involved in the genesis of GHB-induced SWD in this experimental model of generalized absence seizures.

551.6

GABAERGIC MECHANISMS IN THE γ-HYDROXYBUTYRATE (GHB) MODEL OF GENERALIZED ABSENCE SEIZURES. O.C.Snead , Div Neurol, Childrens Hosp Los Angeles, Dept Neurol. Univ South.Calif, Los Angeles 90027

γ-Hydroxybutyric acid is a naturally occurrring compound which has the ability to produce spike wave discharges (SWD) associated with behavioral arrest. The GHB-treated animal thus represents a useful experimental model of absence seizures. Both clinical and experimental absence seizures, including the GHB model, are exacerbated by GABAergic agonists. The purpose of this work was to explore possible GABAergic mechanisms in GHB-induced SWD.

The status of the GABA/BDZ/picrotoxin ionophore was determined during GHB-induced SWD and also in the presence of GHB in varying concentrations in in vitro experiments. GHB had no effect on ^{3}H-muscimol binding , its allostearic modulation by GABA, or ^{35}S-TBPS binding, but did result in a 10-15% decrease in ^{3}H-flunitrazepam binding with no effect on the allostearic modulation of this site by GABA.

At the onset of the SWD induced by GHB there was a significant decrease of ^{3}H-flunitrazepam binding in brain which continued for the duration of the SWD. There was no change of ^{3}H-muscimol binding at the onset of SWD, but one min after SWD onset, the was a significant, albeit transient, increase of ^{3}H-muscimol binding. Allostearic modulation of ^{3}H-muscimol and ^{3}H-flunitrazepam binding by GABA was unchanged throughout the GHB-induced SWD. Scatchard analysis revealed that all significant changes in both the $GABA_A$ and BDZ sites during GHB-induced SWD were due to changes in B_{max}.

These data do not support the hypothesis that the $GABA_A$ site is primarily involved in the genesis of SWD but raise the possibility that the BDZ site might be.

551.7

BIOLOGICAL CONSEQUENCES OF TRANSCRANIAL MAGNETIC STIMULATION IN THE MOUSE. SM Hersch, RC Green, JD Weissman*, HD Rees, KR Davey, CM Epstein* and RAE Bakay, Departments of Neurology and Neurosurgery, Emory Univ. School of Medicine, and Department of Electrical Engineering, Georgia Institute of Technology, Atlanta, Georgia.

Transcranial magnetic stimulation (TCMS) is a recently developed technique for non-invasive stimulation of cerebral cortex which has broad clinical and research applications in humans. However, there is little experimental data on physiologic or pathological effects in animal models. Circular coils are usually used in human TCMS and produce a diffuse field that is not useful for small animal studies. Our high-speed magnetic stimulator utilizes a specially designed U-shaped alloy core electromagnet with a gap of 6 cm and pole faces measuring 2.5 cm2. The induced field reliably stimulates human motor and sensory cortex and is painless as long as the poles do not directly contact the scalp. Male inbred C57BL/6 mice, aged 2-4 months were individually placed into an acrylic restrainer so that the head was within the gap of the U-shaped electromagnet, with the rostral/caudal axis perpendicular to the plane of the electromagnet. Peak electric field at the head position was 2.6 V/cm. Each mouse in the experimental group received a daily total of approximately 1000 stimulations, in three 23-second trains of 15 Hz. Stimulation trains were delivered 1-2 minutes apart over 10-15 minutes. Animals were stimulated daily, 5 days/week for 8 weeks and a total of approximately 40,000 stimulations per animal. Sham-stimulated control mice were placed in an adjacent restrainer outside the curve of the electromagnet exposing them to equivalent noise, but to an electric field less than .01 of that found within the gap of the electromagnet. Behavioral responses were the same in both groups of animals and consisted of a brief startle response and urination. No induced motor activity, weakness, or seizures were identified. Immediately following the final stimulation sequence, experimental and control mice were sacrificed, perfused and processed for histology. Blinded evaluations of Nissl and Fink-Heimer stains did not reveal any differences between experimental and control mice. Further comparisons utilizing electron microscopy and post-stimulation kindling rates are underway.

551.8

SEIZURE REGULATION BY CHOLINERGIC NUCLEI OF THE PONTOMESENCEPHALIC TEGMENTUM. J.W. Miller, M.E. Bardgett and B.C. Gray*. Dept. of Neurology and Neurological Surgery (Neurology), Washington Univ. Sch. of Med., St. Louis, MO 63110

We have previously shown that the central medial intralaminar nucleus of the thalamus (CeM) regulates both arousal and the threshold of seizures induced by chemical convulsants. This nucleus receives direct projections from cholinergic neurons of the pontomesencephalic tegmentum. The present study investigates the effects of injections of GABA agonists into this tegmental region on seizures induced by timed continuous intravenous pentylenetetrazol infusion.

Injections of the direct $GABA_A$ agonist piperidine-4-sulfonic acid (PSA; 5 to 50 nmoles in 50 nl) in the laterodorsal tegmental nucleus (LDTg, cell group Ch 6) depressed arousal and caused a significant ($p<.01$, Student-Newman-Keuls test) reduction in myoclonic seizure threshold (-47%) and clonic seizure threshold (-44%) relative to controls but had no effect on tonic seizures. The $GABA_B$ agonist (-)baclofen (5 to 30 nmoles in 50 nl) had similar effects on arousal and myoclonic (-35%) and clonic (-30%) seizure threshold ($p<.01$), as well as a trend (-16%, NS) towards lowering of tonic seizure threshold. In contrast, injections of PSA (15 nmoles in 50 nl) in pontomesencephalic cholinergic neurons outside the central gray in the vicinity of the pedunculopontine tegmental nucleus (cell group Ch 5) had no effects on spontaneous behavior or seizure threshold.

This study demonstrates that GABAergic mechanisms in the LDTg control seizure threshold and arousal in a fashion nearly identical to the CeM. We hypothesize that the observed effects of LDTg injections are mediated by its ascending cholinergic connections to the CeM.

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551.9

EFFECT OF HYPOXIA ON SEIZURE THRESHOLD AND GROWTH ON NEONATAL RATS. W.H. Hoffman*, M.G. Hoffman*, G.F. Carl, B.B. Gallagher, P.K. Morse* and F. Yaghmi*. Depts. of Pediatr., Neurol. and Pathol., Med.Coll. of Ga. and Medical Research V.A. Med. Center Augusta,GA 30912

We evaluated the effect of hypoxia within the first 24 hrs on seizure threshold and growth in male Sprague Dawley pups. Pups were removed from their mothers and randomly assigned to control (air, 7 hrs, 30°C) and experimental (5% O_2, 95% N_2, 7 hrs, 30°C) groups. Body weight was significantly lower ($p<0.001$, t-test) in the experimental group at 10-150 days. Electroshock seizure threshold difference approached significance ($\bar{x}$ control=26.0 mA, $\bar{x}$ experimental= 20.3 mA, $p=0.08$). Discriminant analysis correctly classified 17 of the 18 rats at day 150, using day 150 weight and seizure threshold as predictors of group. No difference in seizure susceptibility was present for auditory or Indoklon stimuli for the two groups. Liver weight, heart weight, and tail length were significantly lower in the experimental group ($p<0.005$) but brain and testicle weights were not. We conclude that neonatal hypoxia permanently impairs growth and may predispose rats to a greater susceptibility to seizures.

551.10

PERINATAL HYPOXIA HAS LONGTERM EFFECTS ON EEG ACTIVITY AND SEIZURE SUSCEPTIBILITY IN RATS. F.E.Jensen[1], C.D. Applegate[2], AND M. Chansky[1], [1]Children's Hospital Medical and Harvard Medical School, Boston, MA, 02115, and [2]Univ. of Rochester, Rochester, N.Y.

We have shown that hypoxia acutely induces seizures in immature animals but not in adults (F.Jensen, Soc. for Nsci. Abs., 1989). In order to determine whether perinatal hypoxia induces more longterm changes, we exposed 10 day old Long Evans rat pups to hypoxia ($3\%O_2$) ($n=21$) or anoxia ($0\%O_2$) ($n=13$) and then in adulthood (55-65 days old) analyzed baseline EEG spectra and measured the seizure responsiveness to pentylenetetrazol (45mg/kg i.p.). Animals were compared to nonhypoxic littermate controls. During acute oxygen deprivation, the hypoxic group had more epileptiform EEG changes than the anoxic group.

In adulthood, baseline EEG spectra revealed increased slow activity (0-8Hz) in animals that had been hypoxic or anoxic perinatally compared to the controls. Significantly more animals that had perinatal hypoxia had PTZ induced generalized convulsions ($p<0.05$; $X^2=4.22$, $df=1$) than did controls. However, no difference was found between animals that had undergone perinatal anoxia and controls. No significant differences in latency to seizure onset or in seizure duration were found between the hypoxic or anoxic groups and controls.

These results suggest that perinatal hypoxia can have longterm effects on brain activity and seizure susceptibility in adulthood. Perinatal anoxia also appears to cause longterm changes in brain activity, but no effect on seizure susceptibility could be found. We are currently investigating whether blocking acute seizure activity during hypoxia will reduce these longterm effects. (Supported by NIH-NICHD #HD00807 and the Kaplan Foundation).

551.11

MAXIMAL ELECTROSHOCK SEIZURES (MES) IN RATS: ELECTRICAL RECORDINGS FROM CORTEX, FLEXOR AND EXTENSOR MUSCLES AND HEART. J. W. Woodbury and D. M. Woodbury, Dept. Physiol., Univ. Utah Sch. Med., Salt Lake City, UT 84108.

The results are control data for testing the effects of vagal stimulation on MESs. Electrocorticogram (ECoG), electromyograms (EMG) of hindlimb (HL) and forelimb (FL) flexors (Flx) and extensors (Ext), and a chest electrocardiogram (ECG) were recorded on an 8 channnel EEG recorder in awake rats. MESs were initiated by electroshock (150 ma, 60 Hz, 0.2 s). RESULTS. ECoG: Convulsion, 700 µv, 7 Hz spikes lasting 15-20 s, slow fall in size and frequency. Recovery, flat for 20 s; PEDs (paroxysmal epileptic discharges) waxing to 500 µv and waning to zero in 30 s; ECoG normal 90 s later. HL EMG: Flx activity present from onset, lasts 10 s; Ext starts at 2 s, ends at 10-12 s, 5 s before end of seizure in ECoG. FL EMG: Flx appears immediately; Ext starts 1 s sooner and lasts 2 s longer than in HL. ECG: Convulsion: QRS complexes, 2-3 times normal size (bundle branch block?), occur in pairs 0.13 s apart, repeated every 0.8 s. Heart rate (HR) falls from 400 to 150. Recovery. HR rises to 450 at 40 s and falls slowly to normal. ECoG and HR reflect the extreme asphyxia induced by the convulsion.

551.12

IS THE ANTICONVULSANT EFFECT OF NIGRAL INFUSION OF GAMMA-VINYL-GABA (GVG) MODIFIED BY THE GABA A RECEPTOR IN RAT PUPS? S.G.Xu, E.F.Sperber, S.L.Moshé. Depts of Neurology & Neuroscience, Albert Einstein College of Medicine, Bronx, N.Y. 10461

Nigral infusions of GABAergic compounds have provided pharmacological evidence indicating a crucial role for the GABAergic system within the substantia nigra in the control of seizures. Our previous studies showed that nigral infusions of GVG supressed flurothyl-induced seizures in rat pups, while nigrally administratered muscimol (GABA A receptor agonist) or bicuculline (GABA A receptor antagonist) facilitated seizures. In this report, we generated a dose-response curve of the GVG effects and investigated the possible role of nigral GABA A receptors in the mediation of the anticonvulsant effect of GVG in rat pups. Nigral infusions of GVG at a dose ≥ 5 µg significantly delayed the latency of flurothyl seizures compared to controls while doses >10 µg induced sedation. Bilateral nigral infusions of muscimol (100 ng) or bicuculline (100ng) reduced the anticonvulsant effect of GVG.

These findings suggest in rat pups, the optimal dose of intranigrally infused GVG against flurothyl seizures is between 5 and 10 µg/.25µl. There are at least two explanations for the results obtained by the coadministration of the drugs. Since both a GABA A agonist (muscimol) and a GABA A antagonist (bicuculline) had the same effect, one explanation is that the reversal of the GVG effect is due to the algebraic sum of concurrent anticonvulsant and proconvulsant effects. Alternatively, if the muscimol effect is not specific and thus not mediated by the GABA system, our results may suggest that the anticonvulsant effect of GVG may, in part, involve the nigral GABA A receptors.

551.13

IN VIVO ON-LINE ANALYSIS OF CYTOCHROME A,A_3 REDOX STATE WITH EEG AND MICRODIALYSIS IN AN UNANESTHETIZED CAT CORTICAL FOCAL EPILEPSY MODEL. D. Garant*, B. Leheta*, B. Vern*, and T. Bleck. Dept. Neurosci., Rush Med Ctr, Chicago, IL, 60612.

Nine cats received bilateral chronic implants onto the pial surface of the suprasylvian gyrus of an 8mm diam. lens for reflectance spectrophotometry of cytochrome a,a_3. Around each lens were ECoG electrodes and a port for CSF collection; a flow-through dialysis fiber traversed the field. Removable lenses allowed intracortical injections without surgical anesthesia, and easy removal of tissue upon termination.

Unilateral tetanus toxin injection (2.5mm subpial) induced focal epileptiform discharges in all cats by 2-7d postinjection, persisting up to 30d; 5 cats had secondary generalized tonic-clonic convulsions.

Tonic-clonic seizures were associated with profound oxidations of cytochrome ≈15% total reflectance (TR), 3-5x max. oxidation after exposure to 95% O_2/5% CO_2; non-generalizing EEG discharges <3s duration were associated with 2-8%TR oxidations, indicating that even focal epileptiform activity places nontrivial metabolic demands on involved cortex.

Results of biochemical and histopathological analyses pending.

Supported by the Epilepsy Foundation of America.

551.14

AN ELECTRODE TO RECORD SLOW POTENTIAL CHANGES IN DIFFERENT STRUCTURES OF THE BRAIN IN AWAKE ANIMALS DURING CONVULSION AND SPREADING DEPRESSION. F.A.M.de-Azeredo,C.H.Bonfim*,C. Heringer* & J.Fuzimoto*. Laboratorio de Biociências,Universidade Federal Fluminense, CP 100229,Niteroi,RJ,Brazil.

Cortical slow potential(SPC) usually occur during convulsions and spreading depression(SD) but we do not have any information about the occurrence of SPC in subcortical regions.We know that SPC of SD occur in subcortical areas and can remotely influence cell activity at other regions of the brain.We then decided to develop an electrode to record SPC at different brain structures in freely moving animals. The electrode consists of a 1 cm long isolated silver wire with 0.2 mm dia. 1 mm of the tip is peeled off and immersed in 6% NaClO . The Ag/AgCl tip is then coated with a silicone elastomer (elongation rate of 600%).Convulsions were induced in gerbils by ip picrotoxin (PTX) and SD in rats by KCl microinjection.We recorded SPC at cortex,thalamus,striatum and hippocampus and observed SPC in all these regions after PTX and KCl microinjection. The SPC, which is sometimes accompanied by a decrease in EEG activity, reflects a percentage of cells which became refractory to stimulation and will possibly interfere in the firing activity of other cells.Considering the complex cell network we have in the brain we expect that motor and sensory functions will be influenced by the occurrence of SPC in different regions of the brain.

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551.15

SUPPRESSIVE EFFECT OF INTERICTAL SPIKES ON EXPERIMENTAL SEIZURES. Z. Elazar, M. SCHWARTZ* and G.P. Friedmann*. Department of Physiology and Pharmacology, Tel-Aviv University, Tel-Aviv, 69978, Israel

An inverse correlation between the frequency of occurance of the interictal spikes (IS) and of seizures was indicated by previous studies. Other studies did not find any relation between the two epileptic phenomena. We studied this problem in experiments on rats anesthetized with urethane. Penicillin epileptic foci were produced on the cortical somatosensory area. Electrical stimuli (100-500 uA, 1 msec) were delivered to the VPl nucleus. The stimuli strength was adjusted until triggering of the IS was effective. Driving of IS was displayed as a graph in which the Trigger Index (TI = rate of stimulation/frequency of IS) was plotted as a function of the rate of stimulation. The rate of stimulation was increased until TI=1, where no spontaneous IS were recorded and all stimuli were effective. A Suppression Rate (SR) above which seizures were suppressed for the period of stimulation was established for each experiment. The SR was close or above the TI=1. The difference between the SR and the stimulation rate which gave a TI=1 varied with the excitability of
the cortex (depth of anesthesia) and the strength of the thalamic stimuli.

These results suggest that in certain conditions the interictal spike has an inhibitory effect on the mechanism generating the seizure. This inhibitory effect accumulates during trains of IS.

TRAUMA: SPINAL CORD, NMDA AND OTHER

552.1

EFFECTS OF PHYSIOLOGICAL PARAMETERS ON MOTOR AND SENSORY EVOKED POTENTIALS. J.L.Browning, M.L.Heizer*, and D.S.Baskin Dept. Neurosurgery, Baylor College of Medicine, Houston, TX 77030

Motor evoked potentials(MEP) and sensory evoked potentials(SEP) are useful measures for evaluating and monitoring spinal cord and brain function. We therefore studied the effects of temperature, anesthesia, and pCO2 on SEP and MEP.

Cats were anesthetized with halothane(hal) and SEP and MEP recorded and assessed as described by Simpson and Baskin '87. Temp was varied from 42^{0}C to 28^{0}C. Halothane was varied from 0.5 to 2.2%. The CO2 level was adjusted by ventilation rate to paCO2 of 28 to 120.

MEP amplitude(amp) and latency(lat) increases as temp decreases to 28^{0}C. Likewise, decreasing temp causes an increase in SEP lat. SEP amp reveal a parabolic pattern reaching a maximum at 34^{0}C. The level of hal anesthesia had little or no effect on MEP amp or lat. However, SEP amp diminishes and lat increases with increasing hal anesthesia. The effect of paCO2 is to decrease amp and increase lat for both MEP and SEP.

These results suggest that physiological parameters should be closely monitored when making assessments based on MEP and SEP measurements. Additionally, these parameters have different effects on the MEP than the SEP.

552.2

DOSE RESPONSE STUDY OF NIMODIPINE IN ACUTE EXPERIMENTAL SPINAL CORD INJURY. I.B. Ross, C.H. Tator, E. Theriault. Playfair Neuroscience Unit, University of Toronto, 399 Bathurst St., Toronto, CANADA M5T 2S8.

Recent studies of nimodipine treatment in acute experimental spinal cord injury (SCI) in the rat have demonstrated its effectiveness for partially reversing post-traumatic ischemia up to 3 hours after injury. There is also evidence that it improves spinal cord function, as measured by motor and somatosensory evoked potentials. These effects have only been seen when nimodipine is combined with either a vasopressor or a volume expander.

A study being completed in our laboratory at the present time is designed to determine if nimodipine alone, without vasopressor or volume expander, is of any benefit in the treatment of acute experimental SCI in the rat. Thirty minute infusions of .05, .025, .01, .005 or 0 mg/kg of nimodipine after injury are being assessed. Spinal cord blood flow (H2 clearance) and motor and somatosensory evoked potentials are recorded before and after injury and after drug infusion. Preliminary analysis indicates that nimodipine alone is not able to reverse post-traumatic ischemia or improve function as measured electrophysiologically. Supported by Miles Inc. and Rick Hansen Man in Motion Legacy Fund.

552.3

RAPID CLEARANCE OF $[K^+]_o$ FOLLOWING SPINAL CORD CONTUSION IN RAT. M. Chesler, K. Sakatani, Z. Hassan* & W. Young, Dept. of Neurosurgery, NYU Med. Ctr., 550 1st Ave. N.Y. N.Y. 10016.

In cat spinal cord, contusion causes a large increase in extracellular potassium ($[K^+]_o$)(Young, W. et al., Brain Res. 253: 115, 1982). After rising rapidly to a mean of 54 mM, $[K^+]_o$ cleared slowly ($t^{1/2}$ = 45 min.). While rats rather than cats are increasingly used to study spinal injury, comparable data on $[K^+]_o$ are not available. We have therefore studied $[K^+]_o$ following contusion of the rat cord.

Adult Long-Evans rats were anesthetized with pentobarbital, paralyzed and artificially ventilated. Arterial pressure was recorded and sciatic-evoked cortical SEP's monitored. After a T9-11 laminectomy, a 10 gm piston was dropped 5 cm onto the dorsal surface of the cord, with the verterbral column clamped and dura intact. Within 5 min., the dura-arachnoid and pial membranes were incised allowing insertion of a double-barreled K^+-selective microelectrode (valinomycin).

Within 9 minutes of injury, the peak $[K^+]_o$ was 63 ± 9 mM (mean ± SEM, n = 7 rats). In several instances, a gradient of $[K^+]_o$ was noted, the peak value occuring below 500 µm. Compared with previous studies on cats, $[K^+]_o$ cleared rapidly, with a mean $t^{1/2}$ of 12 ± 3 min. The cortical SEP did not recover, despite the return of $[K^+]_o$ to physiologic levels within one hour.

These data demonstrate that after severe spinal contusion, the dynamics of $[K^+]_o$ differ markedly in cat and rat. The rapid clearance in rat may be due to a smaller absolute volume of injured tissue, as well as differences in post-traumatic blood flow. In evaluating mechanisms of secondary injury in the rat cord, our results indicate that $[K^+]_o$ elevation must be considered a relatively transient phenomenon.

552.4

SIGNIFICANT SECONDARY PATHOLOGY IN SPINAL CORD TRAUMA DELAYED BY MORE THAN 24 HOURS. Andrew R. Blight. Ctr. for Paralysis Res., Purdue Univ., W. Lafayette, IN 47907.

Most concepts of secondary pathology in central nervous system trauma have concerned rapid processes of membrane biochemistry, toxin release, edema or ischemia. Evidence of a more delayed damage to axons was described previously from a model of spinal cord contusion in cats (Blight, A.R., *Cent. Nerv. Syst. Trauma* 2: 299, 1985). A functional measurement of such delayed pathology has been possible in a new model of spinal cord injury in guinea pigs. Following lateral compression of the cord at T13 with a specially designed pair of forceps, under ketamine / xylazine / acepromazine anesthesia, animals awoke within a few hours with little or no neurological deficit . From 24-48 hours after injury there was a rapid loss of sensory and motor function below the level of the lesion. Normal hindlimb posture, locomotion and placing responses were lost, together with pain appreciation. The cutaneus trunci muscle reflex of the skin and the free-fall toe-spreading response of the hindlimbs were used to quantify these behavioral deficits over time. Some of the animals recovered most or all of the secondarily lost function within 2-3 weeks after injury. Others remained paralyzed for the 2-3 month duration of the study. The most significant secondary pathology in the guinea pig model appears to be delayed to times well beyond the expected window for numerous proposed mechanisms: lipid peroxidation, lysosomal enzyme or excitatory amino acid release, hemorrhage, edema, ischemia, calcium influx etc. It fits more closely with the time-course of inflammatory responses occurring in the tissue. Similar delays in other injury models may be hidden by some form of "spinal shock". (Supported by grant NS 21122 from NIH/NINDS).

552.5

CHANGES IN BONE FORMATION IN NEONATALLY SPINAL CORD TRANSECTED RATS. V.R. Holets, E. Gunther*, E.L. Hill, C. Cone* and E. Morey-Holton*. The Miami Project, University of Miami, Miami, FL 33136 and NASA-Ames Research Center, Moffett Field, CA 94035.

The mechanism of bone loss following spinal cord trauma is not understood. The purpose of this study was to investigate the rate of bone formation following transection of the spinal cord in neonatal rats that recover some function of their hindlimbs post-lesion. A complete spinal cord transection was done at the T8 spinal cord level in 5 day old rats. Sham lesioned animals served as controls. The rats received alternate (fluorescent) calcein and declomycin injections (s.c.) at 30, 37, 72 and 89 days post-lesion (DPL) to label bone formed during different periods and were sacrificed 90DPL.

Transverse sections (50 um) of tibial diaphyses at the level of the tibiofibular junction were used for bone histomorphometry. Tibial length, medullary area, and cortical area (30DPL and 90DPL) were significantly smaller in lesioned animals as compared to controls. Other bones, above and below the lesion, were evaluated for mechanical properties and trabecular bone histomorphometry. During the period 30-37DPL periosteal bone formation rates were the same in lesioned and control animals (.051 mm2/day), but by 72-89DPL the formation rate in lesioned animals (.013 mm^2/day) was less than that of control animals (.019 mm2/day, $p \leq .01$). This indicates that 1 month after lesion, bone is formed at a normal rate in the tibia. The difference in length and areas of lesioned and control bones 2 months later may be due to an initial lag in growth immediately after lesion that is never compensated for although formation rates return to normal; a decrease in bone formation in older animals, possibly due to secondary changes (neuropathy); or a combination of these. Differences in the cross-sectional shape of the tibiae in lesioned animals indicate that they may use their hindlimbs differently than normal.

552.6

A SENSITIVE FLUOROMETRIC METHOD FOR MEASUREMENT OF VASCULAR PERMEABILITY IN SPINAL CORD INJURY. Z.X. Qu*, J. Xu*, P.L. Perot, Jr.*, N.L. Banik and E.L. Hogan. Depts. of Neurology and Neurosurgery, Med. Univ. of S. Carolina, Charleston, SC 29425-2232

A sensitive fluorometric method was modified for the evaluation of drug action upon rat spinal cord injury. Fluorescein isothiocyanate-labeled dextran (FITC-D, MW 71,200) used as a tracer, was injected IV 2 hr before sacrifice. pH 8.2 was optimal for fluorescence with FITC-D. The recovery in spinal cord was 100.1 ± 4.0% (X ± SD). The extent of FITC-D extravasation was expressed as the vascular injury index (VII).

$$VII = \frac{\text{ng of FITC/mg tissue protein}}{\text{ng of FITC/10 ul of plasma}}$$

and increased in proportion to the trauma force. The VII were 0.16 ± 0.04 (20 g-cm), 0.31 ± 0.03 (40 g-cm), 0.42 ± 0.10 (100 g-cm) and 0.03 ± 0.01 (sham). The peak VII after trauma was at 2 hr. U-50488H (a K opiate agonist) decreased the VII in traumatic cord (0.11 ± 0.03) compared to control (0.16 ± 0.06) at 20 g-cm trauma (X ± SD, n = 6 in each group). This fluorometric method is sensitive, simple and reliable for evaluation of drug effects upon vascular permeability in CNS trauma. Supported by grant NS-11066 from NINDS.

552.7

REGENERATION OF PERIPHERAL NERVES IN PRIMATES THROUGH SEMI-PERMEABLE SYNTHETIC GUIDANCE CHANNELS. M. Goddard*, R. Valentini, J. Cohen and P. Aebischer. Section of Artificial Organs, Biomaterials, and Cellular Technology., Brown University, Providence, RI, 02912.

As part of an effort to develop devices that may improve functional recovery following surgical repair of peripheral nerve injuries, semi-permeable nerve guidance channels (AC) made of the copolymer, poly (acrylonitrile vinyl chloride) were used to repair transections of the recurrent branch of the median nerve in a group of 5 cynomologous monkeys. Nerve transections in one hand were repaired with AC channels and the recovery compared to that of identical lesions on the contralateral side which were repaired with impermeable control channels made of silicone elastomer (SE). Functional recovery was evaluated daily using a timed behavioral paradigm and at the conclusion of the 1 year implantation interval using open electrophysiology. Morphologic evaluation of the regenerated nerve cables was based on myelinated axon counts. Results of the behavioral testing indicated that excellent functional recovery was achieved in this model with both guidance channel designs, however, return to pre-lesion performance levels was faster with the AC devices. The electrophysiology studies demonstrated a mean combined muscle action potential and stimulus threshold of 2.9±2.3 V and 208±53.6 μA respectively for the AC channels versus 2.1± 1.1 V and 252±173.6 μA respectively for the SE channels. Mean regenerated myelinated axon counts were 2476±516 for the AC devices and 1885±727 for the SE devices. The results of this study confirm previous rodent studies which indicated that semi-permeable AC nerve guidance channels show improved regeneration relative to impermeable channels and may hold promise for clinical repair of nerve injuries.

552.8

GLUTAMATE NEUROTOXICITY IN SPINAL CORD CELL CULTURE. R.F. Regan and D.W. Choi. Dept. of Neurology, Stanford Univ. Med. Sch., Stanford, CA 94305.

The neurotoxicity of glutamate was investigated quantitatively in mixed neuronal and glial spinal cord cell cultures from E12 - 13 fetal mice. Five minute exposure to 10 - 1000 μM glutamate produced widespread acute neuronal swelling, followed by neuronal degeneration over the next 24 hr (EC_{50} for death about 100-200 μM); glia were not injured. Glutamate was neurotoxic in cultures as young as DIV 4, although greater death was produced in older cultures. By DIV 14 - 20 approximately 90% of neurons were destroyed by a 5 min exposure to 500 μM glutamate.

Acute neuronal swelling following glutamate exposure was prevented by replacement of extracellular sodium with equimolar choline, with minimal reduction in late cell death. Removal of extracellular calcium enhanced acute neuronal swelling but attenuated late neuronal death. Both acute neuronal swelling and late degeneration were effectively blocked by the noncompetitive NMDA receptor antagonist dextrorphan and by the novel competitive antagonist CGP 37849. In contrast, 100 μM 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) in the presence of 1 mM glycine produced only weak neuroprotection. 7-chloro-kynurenate also inhibited glutamate neurotoxicity, with near complete protection noted at 10 μM; protection was completely reversed by the addition of 1 mM glycine to the bathing medium.

These observations suggest that glutamate is a potent and rapidly acting neurotoxin on cultured spinal cord neurons, and support involvement of excitotoxicity in acute spinal cord injury. Similar to telencephalic neurons, degeneration of spinal neurons induced by brief glutamate exposure is dependent on extracellular Ca^{2+} and the activation of NMDA receptors.

552.9

COMBINED N-METHYL-D-ASPARTATE (NMDA) BLOCKADE AND HYPOTHERMIA (HT) AS A TREATMENT FOR NEURITE TRANSECTION INJURY. G. Wang, J. H. Lucas and G.W. Gross. Center for Network Neurosci., Dept. of Biol. Sci., Univ. of North Texas, Denton, TX 76203.

We have previously reported that temperature reduction to 17°C for 2 h significantly increased the percentage of neurons which survived amputation of a primary dendrite. (Br. Res. 517: 354). Below 17°C, however, both lesioned and uninjured neurons showed dendrosomatic swelling, and, upon rewarming to 37°C, most swollen neurons died (ibid.). We conducted studies to determine: 1) whether swelling during HT involves the NMDA complex, and 2) whether NMDA blockade + HT increases lesioned neuron survival more than HT alone.

Mouse spinal cord (SC) neurons were grown in monolayer cultures. In Study I, 10-15 neurons in each culture were selected for observation. In 3 groups (3-9 cultures/group) the maximum nontoxic concentration of ketamine (100 μM), MK801 (10 μM) or D-APV (30 μM) was added to the medium. No antagonist was added to control cultures. Cultures were kept 2 h at 10°C and 22 h at 37°C. NMDA antagonists minimized swelling during HT. At 24 h only 10% of the selected neurons in each experimental group had died compared to 74% of the controls.

In Study II, a laser microbeam was used to amputate a primary dendrite from each of 10 neurons in each culture (distance from soma = 100 μm). After cell surgery the culture medium was replaced with medium at 10°C, 17°C, or 37°C. D-APV (30 μM) was added to 5 of the 10 cultures at each temperature. Each culture was kept 2 h at its initial temperature, and then 22 h at 37°C. In the groups with initial temperatures of 10°C, 17°C, and 37°C, 24 h survival in the presence or absence of D-APV was 58% ± 10 (SD) vs. 25% ± 13, 66% ± 9 vs. 60% ± 7, and 46% ± 7 vs. 43%± 9 respectively. We conclude that NMDA blockade: 1) protects SC neurons from HT injury, but, 2) compared to HT (17°C) alone, does not significantly increase neuron survival after a transection-type mechanical injury.

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552.10

NEUROTRANSMITTER RELEASE FOLLOWING IMPACT INJURY AND GLUTAMATE NEUROTOXICITY IN THE RAT SPINAL CORD. Danxia Liu and David J. McAdoo. Marine Biomedical Institute, University of Texas Medical Branch, Galveston, TX 77550

Worsening of damage to the spinal cord following initial injury has been attributed to release of damaging substances including neurotransmitters. To investigate this, we sampled released neurotransmitters following impact injury with a dialysis fiber inserted transversely through the rat spinal cord. Glutamate, aspartate, and serotonin, but not norepinephrine, increased substantially following injury. Much of the serotonin detected may come from bleeding. For two hours after impact glutamate and aspartate were estimated to be in a range that can kill neurons. $MgCl_2$ administered through the dialysis fiber reduced the release of the excitatory amino acids, suggesting that part of the ameliorating effect of Mg^{2+} on experimental CNS injury could result from reduced release of excitatory amino acids. NMDA and kainate were administered through the dialysis fiber while stimulating the sciatic nerve and recording field potentials from the dorsal surface of the cord. Responses clearly decreased over 4 h of administration, and histology demonstrated damaged cells.

552.11

PRETREATMENT WITH NMDA ANTAGONISTS LIMITS RELEASE OF EXCITATORY AMINO ACIDS FOLLOWING TRAUMATIC BRAIN INJURY. S. S. Panter and A. I. Faden. Department of Neurolgy, V.A.M.C., San Francisco, CA 94121.

After central nervous system trauma, there are marked elevations in the extracellular levels of excitatory amino acids, which are believed to contribute to delayed tissue damage. Administration of NMDA antagonists reduces injury severity after brain or spinal cord trauma, presumably by blocking the postsynaptic NMDA receptor. In the present studies, levels of extracellular excitatory amino acids were monitored by microdialysis during, and after, a moderately severe (2.8 atmosphere) lateral fluid percussion brain injury to rats, as previously described (Science, 244:798-800, 1989). At the end of the first sampling interval, 10 minutes following injury (n = 6), extracellular glutamate and aspartate increased 20- and 15-fold, respectively, over controls (n = 8). Pretreatment (15 min. prior to injury) with the non-competitive NMDA antagonist dextrorphan (10 mg/kg i.v., n = 4) or the competitive NMDA antagonist CGS 19755 (30 mg/kg i.v., n = 5) significantly attenuated the posttraumatic increase in extracellular glutamate (from 110.9 ± 26.6 (SEM) μM in dialysate from untreated animals to 51.5 ± 7.6 and 64.5 ± 11.2 μM, respectively; $p < .05$ for both groups). Pretreatment with dextrorphan attenuated the posttraumatic increase in extracellular levels of aspartate (15.7 ± 4.3 μM in untreated animals to 10.6 ± 2.1 μM in dextrorphan treated animals); although these differences did not reach significance when examined as absolute values, they were sign when analyzed as percent increase over pre-trauma baseline levels. These results are consistent with recent in vitro experiments and suggest that dextrorphan and CGS 19755 may limit the release of glutamate and aspartate after trauma through mechanisms involving presynaptic modulation.

552.12

THE EXPRESSION OF HEAT SHOCK PROTEIN AFTER BRAIN INJURY IN THE RAT: COMBINED EFFECT OF NMDA ANTAGONIST, VOLTAGE-DEPENDENT CALCIUM BLOCKER, AND A FREE RADICAL SCAVENGER. T. Sanada, L.H. Pitts*, and M.C. Nishimura*. Dept of Neurosurgery, San Francisco Gen'l Hosp and Univ. of Calif., San Francisco CA, 94110.

The pathobiology of head injury has been attributed to a variety of physiological and biochemical events. Given the variety of derangements, we hypothesize that a combination of therapeutic agents may be more efficacious than any single therapy. We examined the effects of combined therapies after brain injury, using the expression of heat shock protein (HSP) as a marker for stressed neurons. Rats were subjected to a temporal fluid percussive brain injury followed by 45 min of hypoxia. Animals were divided into 5 groups: I. saline , II. U74006F (a free radical scavenger) + nimodipine (Ca++ channel blocker), III. U74006F + CGS19755 (an excitatory amino acid antagonist), IV. nimodipine + CGS19755 and V. U74006F + nimodipine+ CGS19755. U74006F (10 mg/kg), CGS (10 mg/kg) or saline were given 10 min (i.v.), and 12 and 24 h (i.p.) after impact. Nimodipine pellets (20 mg) were implanted (s.c.) 10 min after injury. At 72 h post injury sections were prepared for HSP immunocytochemistry , using a monoclonal antibody directed against HSP 72.

The percentage of rats demonstrating HSP 72 immunoreactivity (IRR) in the temporal cortex, parasaggital cortex, caudate-putamen, and hippocampus was calculated for Groups I-V as follows: I- 29, 43, 14, 43, II- 0, 18, 9, 18; III.- 0, 13, 0, 0; IV.- 0, 0, 0, 20; V. 8, 8, 8, 8. Groups III and V showed significantly less IRR in the hippocampus. This preliminary data suggest that combined therapy may ameliorate the expression of heat shock proteins in neurons.

552.13

Intravenous Administration of Heat Shock Protein-70 (HSP-70) Stevens FA*,Gower DJ Section on Neurosurgery and Anatomy, Univ. Of Oklahoma, OKC

Endogenous HSP's are protective for cellular stress. Berbarian et. al 1989 found that exogenous HSP-70 protected cultured cells. We have examined the fate of HSP-70 following intravenous administration in a mammal.

HSP-70 was purified from bovine brain (Schmidt and Rothman 1985). The purified protein was labeled with I-125 and the purity demonstrated by SDS-PAGE. Anesthetized adult rats were administered 25 ug of labeled HSP-70 combined with 10 uCi of TC-99M labeled albumin. Blood and tissue samples were harvested and examined. Initial counts were done in the energy range for the TC-99M. The tissue was allowed to decay for at least 12 half-lives and recounted for I-125.

The albumin space for each organ was determined and the amount of HSP-70 above the amount accounted for by vascular space was calculated. Liver 171.04ng/g,spleen 183 ng/g, kidney 63.33ng/g, and lung26.72ng/g were the only organs to uptake HSP-70. The brain and spinal cord had no selective uptake above vascular space. These data suggest that HSP-70 may be administered intravenously but the usefulness in CNS disease will be limited by an intact blood brain barrier.

552.14

MEASUREMENT OF RHEOLOGICAL PROPERTIES OF INDIVIDUAL NEURONAL CELLS IN RESPONSE TO TRAUMA. T.C Hung*, R.M. Lewis and T.K. Hung. University of Pittsburgh, Pittsburgh, PA 15261.

We are developing a technique for measuring rheological properties of individual neuronal cells. We plan to use this technique to determine how neurons might be affected by mechanical forces, such as those experienced in physical trauma. The technique also will be used to quantify the adhesive properties of neuronal cell adhesion molecules. Dissociated neuronal cells are grown on substrates of varying adhesive properties, or on monolayers of glial cells. The cells are transferred to, or grown in, a chamber which is attached to a pump capable of delivering a controlled flow of solutions of chosen viscous properties. This chamber is placed on a microscope set up for videotape recordings. The shear stress necessary to deform, detach or disrupt individual cells is determined. Cells are seeded onto patches of purified neuronal cell adhesion molecules. The relative adhesiveness of the molecule is determined by the fluid shear force required to dislodge the cell. This technique can be utilized to quantify the adhesive regions of proteolytic fragments or deletion and point mutation products of cell adhesion molecules.

DEGENERATIVE DISEASE—PARKINSON'S: MPTP MONKEYS AND RODENTS

553.1

EARLY BEHAVIORAL AND NEUROCHEMICAL CHANGES CAUSED BY MPTP IN MONKEYS. I. Irwin*, L.E. DeLanney, L.S. Forno, K.T. Finnegan, D.A. Di Monte, and J.W. Langston. California Parkinson's Foundation and California Institute for Medical Research, San Jose, CA 95128.

In this study, the acute evolution of the neurotoxic effects of MPTP were evaluated in the monkey model. Squirrel monkeys (n=17) were given a single subcutaneous injection of MPTP (2.5 mg/kg). The behavioral changes were monitored and the concentrations of dopamine (DA), dihydroxyphenylacetic acid and homovanillic acid were measured in the striatum and substantia nigra 1, 3, 5 and 10 days after drug administration. Neuropathological examination of two animals 8 and 9 days after MPTP revealed severe nerve cell destruction in the substantia nigra. MPTP produced severe parkinsonian behavior changes in all animals after 1 day. Interestingly, although 50-75% reductions were observed in the substantia nigra 1 and 3 days after MPTP, DA was not reduced in the caudate and, actually, was increased in the putamen at these time points. Five and 10 days after MPTP, nigral DA depletion remained greater than 60% of control and striatal DA was reduced 50-85%. At these time points, the putamen was always more affected than the caudate. DA metabolites were decreased in both the substantia nigra and striatum at all time points. These results indicate that: (a) nigral cell bodies may represent an important initial site of MPTP-induced damage; and (b) the interregional pattern of striatal DA deficits caused by MPTP is similar to that seen in idiopathic Parkinson's disease.

553.2

QUANTIFICATION AND TOPOGRAPHY OF MIDBRAIN DOPAMINERGIC NEURONS IN NORMAL AND MPTP-TREATED RHESUS MONKEYS. A.C.Cummins*, K.Bankiewicz, R.Plunkett*. Surg. Neurol. Branch, NINDS, NIH

This study quantifies the tyrosine hyrdoxylase - immunoreactive (TH-IR) cells in the A8-A13 areas of normal and MPTP treated monkeys. Monkeys (n=7) were individually caged and given free access to food and water. Procedures were approved by NINDS Animal Care and Use committee.

Four monkeys received MPTP (0.4 mg/kg by intracarotid infusion) and developed hemiparkinsonism. Four to six months after treatment these and three normal monkeys were euthanized. Brains were cut serially at 25 um and every 15th section stained for TH-IR. Adjacent sections were stained with thionin and for dopamine beta hydroxylase-IR.

TH-IR cells were decreased in MPTP-treated monkeys by 88-96% in A9 and 60-72% in A10. The medial portion of A10 was relatively spared. These cells are likely to represent the population from which sprouting dopaminergic fibers; seen in the caudate of transplanted monkeys, arise. Preliminary data from areas A8 & A11-A13 show no significant depletion after MPTP treatment.

553.3

QUANTITATIVE EFFECTS OF ACUTE MPTP-TREATMENT ON BEHAVIORAL AND BIOCHEMICAL INDICES OF DOPAMINE FUNCTION IN MONKEYS. J.R. Taylor, J.D. Elsworth, R.H. Roth, J.R. Sladek, Jr.†, and D.E. Redmond, Jr. Depts. of Psychiat. & Pharm., Yale Univ. Sch. of Med., New Haven, CT 06510, and † Dept. of Neurobiol. & Anat., Univ. of Rochester, School of Med. & Dent., Rochester, NY 14642

The neurotoxin MPTP produces effects in primates which appear similar to Parkinson's disease. The development of a useful animal model of this disorder requires accurate assessment of the quality, severity, duration of the effects, and complications of this treatment.

Sixty *Cercopithecus aethiops sabaeus* were treated with MPTP (0.3-0.4 mg/kg, 4-5 times, cum. dose 1.2 - 2.4). Trained observers 'scored' and 'rated' behavior twice daily (5 min, 5 x week) for varying periods after MPTP. Scored behavior was one-zero scored (e.g. freeze, immobility, chew, yawn) or scored per 5 sec duration (e.g. eating, drinking). Behaviors were also quantitated on a rating scale of 0-5 (e.g. poverty of movement, limb and head tremor) and after 'challenges' (e.g threat). Individual behaviors were analyzed, as well as summary scores for parkinsonian-, healthy-, anxiety-, and arousal-related behavior. Levels of CSF HVA were determined. In some subjects, performance was also examined on 'executive' or cognitive tasks.

MPTP resulted in behavior not observed in control subjects (i.e., tremor, freezing, immobility, eating problems, delayed and poverty of movement) and reduced signs of healthy, arousal, and anxiety associated behavior. The parkinsonian summary score was used to categorize the severity of MPTP deficits. Identical doses and treatment schedules led to behavioral deficits ranging from extremely mild to severe. All MPTP subjects had reduced levels of HVA in CSF at sacrifice. Medical and physiologic complications analogous to those observed in parkinsonian patients were also observed.

NS24032, Axion Res Found, St Kitts Biomed Res Found, DER - RSA MH400643

553.4

BEHAVIORAL EVALUATIONS OF MPTP-INDUCED HEMIPARKINSONISM IN RHESUS MONKEYS. R.D. Smith*, R.M. Kurlan*, M.H. Kim*, Z. Zhang*, L.A. Cunningham and D.M. Gash.

Department of Neurobiology and Anatomy, University of Rochester, Med. Ctr., Rochester, NY 14642

When induced in monkeys, hemiparkinsonism has been associated with contralateral motor dysfunctions, including bradykinesia and spontaneous rotation. We have developed a diagnostic system to assess the efficacy of hemiparkinsonism as a model for Parkinson's disease (PD). Multiple clinical evaluations were done over 4-5 months before and after MPTP was administered unilaterally to 4 adult female rhesus monkeys. Completeness of MPTP lesions was indicated by apomorphine-induced rotations and behavioral impairment. Monkeys were observed continuously using computer-assisted, infrared activity monitors. For the course of the experiment, the amount of activity changed in only one animal (up 40% due to spontaneous rotation after lesion). A 24-item rating scale developed to assess parkinsonian features (eg., tremor) and general behavioral responses (eg., grooming) was used to monitor the course of impairment. Ratings indicated that the maximal effect of the lesion occurred at 10-15 days. Not all monkeys demonstrated the full range of symptoms characteristic of hemiparkinsonism. Impairment was variable (range=52-91%) and the severity of parkinsonism diminished by 27+/-17%. This partial recovery occurred within 20 days and had stabilized at 8 weeks. The use of an afflicted limb often varied with the type of challenge. Thus, symptoms routinely associated with hemiparkinsonism in non-human primates reflect only some of those symptoms normally associated with PD.

Supported by NIH grant NS 25778 and the PEW Foundation.

553.5

EFFECTS OF LOCUS COERULEUS LESIONS ON MPTP-INDUCED PARKINSONISM IN SQUIRREL MONKEYS. Mihalis Mavridis*, A.J. Lategan*, A.-D. Degryse*, M.R. Marien and F.C. Colpaert, FONDAX-Groupe de Recherche SERVIER, 92800 Puteaux, France.

Female squirrel monkeys (7 pairs, 550-750 g) received daily i.p. injections of n-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP; 0.63-1.25 mg/kg) for 9-14 days, beginning on the day of either a bilateral 6-hydroxydopamine lesion of the locus coeruleus (LC) or a sham operation. Seven to nine weeks after the start of MPTP treatment, and in marked contrast to the sham-operated animals, the LC-lesioned monkeys showed a profound decrease in striatal dopamine levels (>84% depletion), extensive cell loss in the substantia nigra, and little or no recovery from the parkinsonian motor symptoms (tremor, bradykinesia, hypokinesia and reduced blink rate) induced by MPTP.

These neurological, biochemical and histological assessments indicate that lesioning of the LC impairs the recovery which typically occurs from the parkinsonian manifestations induced by MPTP in squirrel monkeys. Results of this study support the hypothesis (F.C. Colpaert, Neuropharmacol. 26: 1431, 1987) that deficient LC-noradrenergic mechanisms might play an underlying role in the progression of Parkinson's disease.

553.6

EFFECTS OF D1 AND D2 AGONISTS AND ANTAGONISTS ON DYSKINESIA PRODUCED BY L-DOPA IN MPTP-TREATED MONKEYS. B.G. Mancilla, R. Boucher, and P.J. Bédard. Lab. of Neurobiology, Univ. Laval and Hosp. Enfant-Jésus, Québec, CAN. G1J 1Z4.

In order to study pharmacologically the action of D_1 and/or D_2 receptor agonist on dyskinesia produced by L-DOPA, we performed the following experiment. A group of 5 female cynomolgus monkeys (±3kg) was rendered parkinsonian by one or repeated doses of MPTP (0.3-10 mg/kg). At least two months after the last injection, a daily oral treatment with L-DOPA/carbidopa 150/37.5 mg was begun and continued for two months. All animals showed evident dyskinesia mainly in the lower limbs starting at 6 weeks of treatment. After two months, several days a week the daily dose of L-DOPA was replaced by an experimental agent and the animal monitored for dyskinesias and other effects. All D_2 agonists (dose in brackets in mg/kg) reproduced the same dyskinesia as L-DOPA, Quinpirole (0.01, 0.1), PHNO (4μg), Bromocriptine (5).

The partial D_2 agonists terguride (5) and -3-PPP (5) also reproduced the same dyskinesia. The D_1 agonist SKF (5) was without effect and so was the D_1 agonist CY208243 at dose of 0.1. However a dose of 0.5 mg/kg of CY208243 induced some dyskinesia.

The D_1 antagonist SCH23390 (0.05) as well as th D_2 antagonist Sulpiride (30) were without effect by themselves. However, dyskinesia induced by Quinpirole could be antagonized by SCH23390 as well as by Sulpiride and prevented by previous treatment by alpha-methyl-paratyrosine. Our results show that although dyskinesia are mediated mainly by D_2 receptors, D_1 receptor activation appears necessary for their manifestation. Supported by MRC of Canada and Parkinson Foundation of Canada.

553.7

PHARMACOLOGICAL MANIPULATION OF COGNITIVE PERFORMANCE AND IMPERSISTENCE IN MOTOR ASYMPTOMATIC MONKEYS CHRONICALLY EXPOSED TO MPTP. C.J. Kovelowski, II and J.S. Schneider. Dept. of Neurology, Hahnemann University School of Medicine, Philadelphia, PA. 19102.

Monkeys exposed to low doses of MPTP over several months have been shown previously to develop specific cognitive deficits in the absence of overt motor disturbances. In the present study, three M. nemistrina monkeys were trained to perform delayed response (DR), delayed match-to-sample (DMS), visual discrimination (VD), object discrimination reversal (ODR), and object retrieval (OR) tasks. Thus far, monkeys have received up to 4 months of MPTP treatment (0.05-0.15mg/kg, i.v.) given 2-3 times/wk. In all animals, DMS was the task most sensitive to disruption. In time, performance of ODR, OR, and DR was also disrupted. In contrast, VD performance has remained intact. In addition to performing the previously learned tasks incorrectly, animals demonstrated striking impersistence during attempted task performance with many incomplete trials recorded. On the object retrieval task, after numerous MPTP injections, animals consistently failed to retrieve food that was not readily accessible via direct line of sight. These animals continue to do poorly and fail to make appropriate detour movements even when shown how by the experimenter. Preliminary pharmacological studies have shown that the dopamine D2 agonist LY-171555 and to a lesser extent, the nonspecific agonist apomorphine reduce the impersistence in these animals. While monkeys can complete the trials more consistently under these drugs, their cognitive performance is not improved. The partial D1 agonist SKF-38393 caused a slight but insignificant improvement in the impersistence and also did not help cognitve performance. Clonidine, an alpha 2 adrenergic agonist significantly improved DR performance. The effects of other drugs will also be discussed. The results thus far suggest that the mechanisms responsible for impersistence and the correct performance of the cognitive tasks may be different. Supported by the Alzheimer's Disease and Related Disorders Association, Inc.

553.8

6-[^{18}F]FLUORO-L-DOPA METABOLISM PARALLELS DOPAMINERGIC SYSTEM CHANGES IN MPTP-TREATED MONKEYS. W.P. Melega, J.S. Schneider, M.E. Phelps, and J.R. Barrio*. UCLA School of Medicine, Los Angeles, CA 90024

6-[^{18}F]Fluoro-L-DOPA (FDOPA), an L-DOPA analog, is used with positron emission tomography (PET) for the in-vivo assessment of the functional integrity of presynaptic dopaminergic mechanisms in humans. The significance of these studies is, however, dependent upon the demonstration that the biochemistry of FDOPA can be correlated with that of the endogenous dopamine system. In the present work, two unilateral MPTP-treated monkeys and three controls, pretreated with carbidopa (5 mg/kg, i.v.) were administered FDOPA (5-8 mCi, i.v.) and sacrificed 60 min later. Putamen, caudate, frontal cortex , and cerebellum were dissected and analyzed by HPLC for endogenous catecholamine and FDOPA metabolite levels. For both MPTP treated monkeys, lesioned side dopamine (DA) and 6-[^{18}F]fluorodopamine (FDA) levels were reduced by at least 98% when compared with controls; DA and FDA metabolites were also significantly reduced. The homovanillic acid (HVA):DA ratio, an index of dopamine turnover, increased to 7:1 from control values of 1.9:1. Similarly, the 6-[^{18}F]fluorohomovanillic acid (FHVA): FDA ratio increased to 6.4:1 from 0.34:1 (controls). In one MPTP-treated monkey that also sustained a partial contralateral lesion, DA and FDA levels were reduced 84 and 81% respectively; metabolite levels and turnover ratios were intermediate between those of controls and total lesions (HVA:DA 4.3:1; FHVA:FDA 1.4:1). These results indicate that analysis of human PET studies with kinetic models to distinguish FDA storage from clearance can provide evidence of DA turnover changes in vivo.

553.9

EFFECTS OF MPTP ON ENKEPHALIN-, SOMATOSTATIN- AND SUBSTANCE P-CONTAINING NEURONS IN MOUSE BRAIN. K.Mitsuo*, J.D.Harvey-White* and J.P. Schwartz. Clinical Neuroscience Br., NINDS, NIH, Bethesda, MD 20892

We have examined the effects of MPTP on three neuropeptides, met-enkephalin (ME), somatostatin (SS) and substance P (SP), which have been observed to change in Parkinsonian brains. MPTP (4 x 24 mg/kg i.p., MPTP-HCl) was given to young adult C57Bl mice. Mice were sacrificed 1 day to 6 wks later and dopamine(DA) and its metabolites (DOPAC, HVA), as well as the peptides and their precursor mRNA levels were measured in striatum (ST), olfactory tubercle (OT) and prefrontal cortex (PF) of the same animal. DA content was depleted 70% in ST and 40% in OT with no change in PF except for a decrease of HVA. ME increased in all three regions. Proenkephalin mRNA increased in OT, decreased in PF,and decreased at 1 day but increased at 3 days in ST. Both SS and SS mRNA increased in PF. SP mRNA decreased in ST. These data suggest that MPTP can differentially affect these neuropeptides. The discrepancy between the expected effects due to DA loss and the actual changes observed suggest that MPTP can affect these neurons not only through DA loss but also by some direct action.

553.10

CHANGES IN DOPAMINE (DA) RELEASE IN VITRO FROM THE CORPUS STRIATUM (CS) OF YOUNG AND AGED RATS AS A FUNCTION OF INFUSION MODE OF L-DOPA, K^+ AND AMPHETAMINE (AMPH). J. McDermott, D. Dluzen, and V. Ramirez. Departments of Medicine-Carle Hospital and Physiology and Biophysics, University of Illinois, Urbana, IL 61801.

We have reported differences in DA release in vitro from the CS of young versus aged rats in response to a pulse infusion of L-DOPA (Dluzen, et al. Exp. Neurol., 106:259, 1989). In the present experiment, CS tissue fragments from young (Y:2-4 months) and aged (A:18-24 months) male rats were superfused in vitro and received either two 10 min pulses (80 min inter-pulse interval) or continuous (120 min) infusions of L-DOPA (5μM), K^+ (30 mM) or AMPH (10μM). For both young and aged rats the peak response ratios of the second/first response for the two infusions were virtually identical for K^+ (Y=0.3±.07, N=4 vs A=0.42±.05, N=7) and AMPH (Y=0.46±0.8, N=4 vs A=0.46±.03, N=6). However, there was a significant difference ($p<.005$) in response to L-DOPA pulse infusions (Y=2.48±0.33, N=7 vs A=1.24±0.17, N=7). Overall DA release to continuous infusions ($\bar{x}$±SEM-pg/120 min) were greater in young vs aged CS for K^+ (Y=84.6 ±18.7, N=4 vs A=21.2±3.5, N=6) and AMPH (Y=97.3±13.2, N=4 vs A=52.5±8.5, N=6). In contrast, a continuous infusion of L-DOPA resulted in a greater release of DA from aged CS (Y= 258.2±46.3, N=7 vs A=410±45.4, N=6). These results demonstrate infusion mode dependent differences in DA release from the CS of young versus aged rats in response to these secretagogues.

553.11

VITAMIN E DEFICIENCY IN 6-OHDA SUBSTANTIA NIGRA LESIONED RATS. B.I. DIAMOND, E.A. O'NEAL*, S.D. SMITH*, J. WANG*, and J.L. THOMPSON. Psychiatry Dept., Med. Coll. of Georgia, Augusta, GA 30912.

The role of vitamin E (VIT E) deficiency on striatal dopamine (DA) system function was studied in rats. 6-OHDA unilateral lesions of the substantia nigra (SN) were induced in 20 male Sprague-Dawley rats (250-300 g) which were then tested 14 days later for turning behavior in response to i.p. injections of the DA agonist, apomorphine (APO), 1mg/kg, and the indirect agonist, d-amphetamine (AMP), 3.5 mg/kg. Rats were assigned to either a normal or a VIT E deficient diet. They were challenged weekly for 9 weeks with APO, followed in 48 h by AMP. Completed turns were counted. We hypothesized that since neurons in the SN undergo loss with aging, VIT E deficiency would result in an even more rapid loss due to oxidative damage and that this would result in a more rapid decrease in turning rate. However, there were no group differences in the turning rates, although VIT E deficient rats exhibited a trend towards increased turning in response to AMP ($p=0.12$). This result is consistent with our other findings of significantly increased striatal DA levels in rats on a VIT E deficient diet.

553.12

INHIBITION OF MITOCHONDRIAL RESPIRATION BY N-METHYLATED BETA-CARBOLINE ANALOGS OF MPP+. M.A. Collins, E.J. Neafsey, R. Albores*, G. Drucker, and J.Z. Fields. Depts. of Biochem., Anatomy, and Pharm., Loyola Univ. of Chicago Stritch Sch. of Med. Maywood, IL 60153.

Since the 2[N]-methyl-beta-carboline (2Me-BC), 2Me-harmine, approaches MPP+ in potency of inhibition of mitochondrial respiration in vitro (Hoppel et al., 1987), we have examined a number of physiologically possible 2Me-BCs and dihydro-BCs (2Me-DHBCs) for their effects on O_2 utilization by rat liver mitochondria. Following 6 min pre-incubations, 2Me-harmine, 2Me-harmol, and 2Me-harmaline had IC50's comparable to MPP+, whereas 2Me-norharman and 2Me-harman were only weak inhibitors. Like MPP+, inhibition by 2Me-DHBC/BCs was augmented by tetraphenylboron and reversed by DNP, consistent with the involvement of cationic 2Me-DHBC/BC forms. However, the inhibitory time courses and DNP uncoupling patterns were dissimilar from MPP+, indicating a role for uncharged 2Me-DHBC/BC forms as well. The neutral 2Me-BC tautomers may arise from deprotonation of the indole N, since 2,9-dimethyl-norharman, a cationic BC that cannot deprotonate, had an inhibitory time course and response to DNP that resembled MPP+ rather than other BCs. Also unlike MPP+, the 2Me-DHBC/BCs blocked succinate-supported respiration almost as effectively as NAD+-linked respiration. The findings are consistent with the hypothesized role of endogenous 2Me-DHBC/BCs in the etiology of Parkinson's disease. (Support: NIH NS23891)

553.13

INDOLE METHYLATION OF 2-METHYL BETA-CARBOLINES DRAMATICALLY INCREASES THEIR TOXIC EFFECTS IN PC12 CELLS. R.J. Cobuzzi, Jr., E.J. Neafsey and M.A. Collins, Depts. of Biochem. and Cell Biology, Neurobiology and Anatomy, Loyola Univ. Stritch Sch. of Med., Maywood, IL 60153

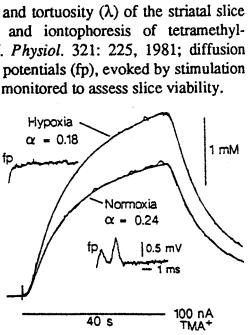
In ongoing studies of the neurotoxic capabilities of MPP+-related beta-carbolines (BCs), we have found that the PC12 cell cytotoxicity exerted by cationic 2[N]-methyl BCs is significantly enhanced by further methylation of the indole-9 nitrogen. This was suggested by initial results with isolated mitochondria in which 2Me-norharman, a very weak inhibitor of respiration, became more effective than MPP+ when the 9-N proton was replaced by methyl. Previously in PC12 cells, we observed that 2Me-norharman produced negligible release of LDH or decrements in cell protein and dopamine uptake (indicators of cell toxicity) in 4 day incubations. In contrast, 2,9-diMe-norharman was equipotent with MPP+ in all three measurements after 2 days. Similarly, 2,9-diMe-harmine was identical with MPP+ and 2,9-diMe-norharman, whereas 2Me-harmine showed minimal toxicity at 2 days. A 3rd compound, 2,9-diMe-harman, achieved toxicity equal to MPP+ at 4 rather than 2 days, while the monomethylated 2Me-harman was without effect throughout. If metabolically feasible, replacement of the indole proton with a methyl or other group may be an effective means of enhancing the neurotoxicity of endogenously-derived 2Me-BCs. (Support: NIH NS23891).

553.14

EXTRACELLULAR VOLUME FRACTION AND DIFFUSION CHARACTERISTICS OF THE RAT STRIATAL SLICE DURING NORMOXIA AND HYPOXIA. M.E. Rice & C. Nicholson. Dept. Physiology & Biophysics, New York University Medical Center, New York, NY 10016.

Replacement therapies for Parkinson's disease often rely on the diffusion of dopamine (DA) or related compounds into the striatum. Surprisingly little is known about the diffusion characteristics of this region, however. As a prelude to further studies of DA diffusion in the striatum (Rice et al. *Neuroscience* 15: 891, 1985), we evaluated the extracellular volume fraction (α) and tortuosity (λ) of the striatal slice using ion selective microelectrodes (ISMs) and iontophoresis of tetramethylammonium (TMA^+) (Nicholson & Phillips, *J. Physiol.* 321: 225, 1981; diffusion curves illustrated in Figure). Extracellular field potentials (fp), evoked by stimulation of afferent fibers from the cerebral cortex, were monitored to assess slice viability.

Under normoxic conditions (superfusion with saline equilibrated with 95% O_2 / 5% CO_2), α was 0.21 ± 0.04 and λ was 1.57 ± 0.13 (mean ± SD; n = 170). Under hypoxia (saline with 95% N_2 / 5% CO_2 for 10-20 min), evoked field potentials were reversibly eliminated, while α decreased by 30% to 0.14 ± 0.04 and λ rose slightly to 1.61 ± 0.23 (n = 23). In normal media, α and the quality of evoked fields were correlated; α was 0.10-0.18 in slices with small post-synaptic fields. These results demonstrate the sensitivity of brain extracellular volume to tissue condition.



Supported by NINDS NS-13742 and the American Parkinson Disease Association.

553.15

NEUROTENSIN RECEPTORS ON DOPAMINERGIC NEURONS:EVIDENCE ON CELL CULTURES AND APPLICATION FOR FETAL TISSUE TRANSPLANT VISUALIZATION

W.Rostene,Y.Masuo*,D.Scherman*,JP.Herman* and D.Pelaprat*.INSERM U.55 Hop. St Antoine 75012 Paris;CNRS Institut Curie Paris; INSERM U.259 Bordeaux, France.

We previously demonstrated on tissue sections Neurotensin (NT) binding sites located on dopaminergic (DA) cell bodies and dendrites in the rat mesencephalon. We show here the presence of NT receptors on rat mesencephalic DA neurons in culture, by a double labeling procedure based on 125I-NT receptor binding autoradiography coupled with tyrosine hydroxylase (TH) immunocytochemistry. NT binding was first followed by a glutaraldehyde fixation, secondly by an immunohistochemical procedure and finally by an emulsion step. Light microscopic observation demonstrated that 80% of the TH-positive cells were endowed with NT receptors, the latter being distributed on both cell bodies and processes. We thus used 125I-NT as a marker of fetal mesencephalic cell suspension grafted into the striatum of previously 6OHDA-lesioned rats. Three months after graft, rats which recovered normal behavior showed 125I-NT binding located at the periphery of the graft where TH cells are found. Moreover, on adjacent sections, 3H-dihydrotetrabenazine binding, a marker of DA nerve terminals integrity (Masuo et al, Neurosc. Lett, in press), allowed us to follow the reinnervation of the host striatum by the graft processes. These two ligands can thus be used to label DA pathways and follow nerve degeneration or reinnervation in various experimental and pathological diseases such as Parkinsonism. Supported by INSERM, Canon Foundation and Fondation de France.

553.16

EXCESSIVE METHYLATION AND PARKINSON'S DISEASE: MODULATION OF DOPAMINE. B.Crowell,Jr. and C.Charlton. Dept. of Physiology, Meharry Medical College, Nashville, TN 37208

Catecholamine neurotransmitters, eg. dopamine (DA) are metabolized by the enzyme catechol-O-methyltransferase (COMT) which uses S-adenosyl-methionine (SAM). When SAM is injected into the lateral ventricle of rats tremors, hypokinesia and abnormal posture are observed. These impairments are also observed in Parkinson's disease (PD), along with a decrease in DA. Therefore, SAM may play a role in PD. We tested this hypothesis by manipulating the levels of SAM in the brain of rats and observed changes in motor performance and levels of DA in the caudate nucleus (CN).

Rats (150-200gm) were cannulated and 1.88 and 0.65 umol of SAM in 5 ul of phosphate buffered saline (PBS) administered intraventricularly. Controls recieved 5 ul of PBS. The rats were briefly observed and sacrificed 15 min after injection. DA was determined using radioenzymatic assay and HPLC.

SAM produces tremors, abnormal, hypokinesia, etc. The onset was 1-2 min. SAM decreased DA levels in the CN by 34.1 and 31.5% in animals receiving 1.88 and 0.65 umol of SAM respectively. In comparing the ipsilateral with the contralateral CN the 1.88 umol dose reduced DA by 45.9 and 25.4%, the 0.65 umol dose by 49.1 and 9.5%. These results complement our previous finding of SAM-induced depletion of tyrosine hydroxylase in the CN.

Motor disorders caused by SAM are typically those that also occur in PD. DA is also decreased in the brain of PD patients. Since SAM is involved in the metabolization of DA it is possible that SAM may play a role in PD.

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553.17

EXCESSIVE METHYLATION AND PARKINSON'S DISEASE: L-DOPA EFFECTS. R. Benson, J. Clark, C. Charlton. Dept. of Physiology, Meharry Medical College, Nashville, TN 37208.

When injected into the brain of rats and mice, S-adenosyl methionine (SAM) causes Parkinson's disease (PD)-like symptoms, such as tremors, dyskinesia, and abnormal movements. The major treatment for PD is l-dopa. L-dopa is metabolized to dopamine (DA) and reacts with SAM. Therefore, the increase in DA as well as the depletion of SAM may elicit the positive benefits of l-dopa therapy. It follows that the lack of efficacy from prolonged l-dopa therapy may be due to a rebound increase in SAM via activation of methionine adenosyl transferase (MAT), the enzyme that synthesizes SAM from methionine and ATP. We tested this hypothesis by administering (i.p.) saline, or l-dopa to rats and mice and assaying for MAT activity.

About 73% and 53% of the activity of MAT was observed in the insoluble fraction of the brain and liver, respectively. L-dopa increased the activity of MAT in the brain by 32% and 37% in the 100 and 500 mg/kg, as compared to saline, treated rats. L-dopa, 50 mg/kg, increased the MAT activity by 37% in mouse brain. No significant changes occurred in the liver of rats or mice. Since l-dopa increases MAT, the decreased efficacy of chronic l-dopa in PD therapy may be explained partly by an increased MAT activity, which will increase SAM and create a vicious cycle. SAM will react with both l-dopa and DA to produce methylated products. It has been shown that high plasma levels of methyldopa were observed in patients exhibiting l-dopa induced dyskinesia; also, in animals, methyl-DA and SAM caused motor deficits antagonistic to l-dopa.

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553.18

A MONOCLONAL ANTIBODY (MAb) TO DARP (DOPAMINE RELEASING PROTEIN) SELECTIVELY KILLS NEURONS IN CULTURE FROM THE MESENCEPHALON AND REDUCES DOPAMINE (DA) LEVELS IN THE STRIATUM (CS) AND HYPOTHALAMUS OF IMMATURE RATS. V.D. Ramirez, D.E. Dluzen, S. Kuhananthan, S. Miklasz* and F. Marcus*, Department of Physiology, U of IL and Chiron Corp.

Cell cultures from mesencephalon (M) and diencephalon (D) of 17-18 day-old rat embryos were prepared. On day 9, substances dissolved in minimal essential medium were added. On day 16, cells were counted. A dramatic arrest of growth and neural differentiation of M cultures that received the MAb (14 μg/well) was observed. The numbers of neurons/1.7mm^2 were: 14.6±1.4, n=27 controls vs 2.0±0.5, n=24 MAb-treated. In the D cultures were: 8.1±1.7 n=9 control vs 6.8±1.3, n=12 MAb-treated. Recent born pups were injected subcutaneously daily for 10d with MAb-E$_2$ 20 or 5 μg/rat or with 20 μg γ-globulin. On day 11 or 25 DA levels were measured in the CS or hypothalamus. The MAb decreased DA concentrations in the CS on day 11 (3.9±0.26, 3.7±0.19 and 2.6±0.16 ng/mg, n=5-6 for controls, 5 μg and 20 μg groups, respectively) and in the hypothalamus on day 25 (840±70 controls vs 482±61 pg/mg 20 μg/MAb-treated, n=5). These data suggest that an endogenous neurotrophic factor is released from cells of M cultures that is essential for their growth and neuronal differentiation. The anti-DARP MAb blocks this action and is also capable of reducing DA levels from the CS and the hypothalamus.

DEGENERATIVE DISEASE OTHER: MS, ALS AND OTHERS

554.1

COGNITIVE-MNESTIC INFORMATION PROCESSING IN MULTIPLE SCLEROSIS PATIENTS. H.J. Markowitsch+, P. Calabrese+, M. Haupts+ and W. Gehlen+ (SPON: ENA), Lab. of Biopsychology and Knappschafts-Hospital, Ruhr-University, D-4630 Bochum, Federal Republic of Germany.

Hospitalized patients with multiple sclerosis were given a variety of psychometric tests with emphasis on cognition and memory. Prior to neuropsychological testing, all patients had been intensely scrutinized neurologically and neuroradiologically, including MR-imaging for some of them. Aside from an intelligence test we used a modified form of the Rivermead Behavioural Memory Test, and further tests on nonverbal memory span and problem solving abilities. In general, we found - compared to non-brain damaged subjects - a deterioration on most of the intellectual abilities tested. The poor performance was, however, less obvious for verbal memory as opposed to conceptual-intuitive and visuo-spatial abilities. Performance on the Corsi-cube test, for instance, was not only worse than in Korsakoff subjects, but also worse than in amnesics with profound damage of limbic-system related structures. We conclude from our results that multiple sclerosis patients even at an early stage of the disease process need intensive neuropsychological testing and might profit from continuous cognitive rehabilitation training and/or guidance.

554.2

HIGH EXTRACELLULAR CALCIUM INCREASES CONDUCTION IN EXPERIMENTALLY DEMYELINATED FIBERS OF RAT FIMBRIA. K. H. Reid and J. Newmark*. Depts. of Anatomical Sciences and Neurobiology and of Neurology, Univ. of Louisville and Louisville VA Medical Center, Louisville KY.

Using a previously developed in vitro model of acute CNS demyelination (stereotaxic injection of 0.3 ul of lysophosphatidyl choline 7 days pre-test), we exposed partially demyelinated rat fimbrias to artificial cerebrospinal fluid containing [Ca^{++}] of 2.5 mM (physiologic), followed by alternations between 4.4 mM (high) and 0.25 mM (low). We recorded population spike amplitudes both of the normal peak, which we associate with undamaged fibers, and of the delayed peak, which we associate with partially demyelinated fibers. In high [Ca^{++}] both peaks increased in amplitude, while in low [Ca^{++}] both decreased in amplitude. The effect was reversible and was not seen in control (undamaged) fimbrias. This effect is consistent with a model of membrane stabilization by calcium ions which has been proposed as a basis for dietary prevention of colon cancer. Our findings suggest that increased extracellular [Ca^{++}] may be protective in acute exacerbations of multiple sclerosis. Supported by a grant from the Neurology and Neurobiology Merit Review Board, U.S. Dept. of Veterans Affairs.

554.3

IMMUNOLOGICAL REACTIONS IN AMYOTROPHIC LATERAL SCLEROSIS BRAIN AND SPINAL CORD TISSUE. T. Kawamata*, H. Akiyama, T. Yamada, P. L. McGeer, E. G. McGeer. Kinsmen Laboratory of Neurological Research, University of British Columbia, Vancouver, B.C. Canada, V6T 1W5.

Expression of proteins associated with immune function was investigated immunocytochemically in postmortem brain and spinal cord tissue from patients with amyotrophic lateral sclerosis (ALS). These included the leukocyte surface glycoproteins LCA, CD4, CD8, FcγRI, FcγRII; complement receptors CR3 and CR4; complement proteins C3d and C4d; LFA-1 and its ligand ICAM-1; and HLA-DR. Reactive microglia expressed high levels of LCA, FcγRI, FcγRII, CR3, CR4, LFA-1 and HLA-DR. They were found in abundance in the primary motor cortex, motor nuclei of the brain stem and the anterior horn of the spinal cord. Throughout the corticospinal tract from subcortical white matter of the motor cortex to the anterior and the lateral funiculi of the spinal cord, the cells were lipid filled having the morphology of classical fat granule cells. A significant number of lymphocytes positive for LCA, CD4 and CD8 were observed marginating along the walls of blood vessels and invading the parenchyma of inflamed areas. Clusters of C3d and C4d coated fibres were frequently associated with oligodendroglia. We describe these as complement-activated oligodendroglia. Labelling of the central nervous system components with complement proteins might be evidence of opsonization, which could stimulate phagocytosis by reactive microglia/macrophages bearing complement receptors. Expression of HLA-DR by these phagocytes indicates their capability of antigen presentation to T-lymphocytes, possibly followed by the production of specific antibodies.

554.4

ISOLATION OF MOTONEURONS AND CHARACTERIZATION OF RNA AND NEURONAL INTERMEDIATE FILAMENT PROTEINS FROM AUTOPSY VENTRAL SPINAL CORDS OF PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS. B.A. Brody, D.C. Kelley-Geraghty* and B. Jubelt*. Northwestern Univ. Sch. of Med., Chicago, IL 60611.

A variety of methods have been utilized to isolate large numbers of neurons from the brains of adult animals or from human autopsy material. A quantitative analysis of motor neuron RNA has been reported for bovine spinal cord (Capps-Covey, P. and McIlwain, D.L., J. Neurochem., 25:517, 1975). The resulting membrane defects caused by mechanical dissociation of neurons as well as postmortem autolysis make obtaining intact neurons from human autopsy tissue particularly difficult. We are able to obtain highly enriched fractions of neurons from the ventral horns of frozen spinal cords from both patients with amyotrophic lateral sclerosis (ALS) and control patients with nonneurological disease. The neuronal yield from ALS patients is approximately 1/10 that of the controls. We have been successful with autopsy material with as much as an 18 hour postmortem interval or frozen for as long as 4 years. We describe immunoperoxidase and immunofluorescence studies on these neuronal fractions utilizing antibodies to high molecular weight and to 57kDa neurofilament proteins. We have also quantitatively isolated nondegraded RNA from frozen ventral spinal cord tissue of both ALS and neurologically normal patients. These studies will provide a basis for biochemical, immunological, and biological characterization of ALS versus normal tissue.

554.5

CELLULAR CHANGES IN THE MOTOR CORTEX OF PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS. E. J. Kasarskis, H. Liu*, H. Wang*, and S. Khare*. Dept Neurol, VA and Univ of Kentucky Med Ctrs, Lexington KY 40536-0084.

Amyotrophic lateral sclerosis (ALS) is characterized by selective death of motor neurons in the spinal cord. Pyramidal tract involvement and pathological changes in the motor cortex are also recognized. However the extent of neuronal loss in precentral cortex has not been quantitated.

Four patients dying with ALS and 4 age-matched controls were studied. A series of images, comprising a "core" of cortex taken from the leg region of Broadmann's area 4 and measuring 270 x 3400 sq microns, were analyzed by a computerized image analysis system. A total of 4 cores were examined from each subject. The apparent area and position of each cell relative to the pial surface was determined.

The total number of cells increased by 19% in ALS cortex, reflecting primarily a 30% increase in the number of glial cells. However neurons measuring >100 sq microns, were reduced by 50% in lamina V of ALS cortex.

These data indicate that significant neuronal loss occurs in lamina V of Broadmann's area 4 in ALS. Changes of similar magnitude, if present in other cortical regions which contribute to the pyramidal tract, may explain the cortical hypometabolism seen in PET scanning of ALS patients. (Supported by NS 25165).

554.6

Tubulin Distribution is Altered in Spinal Cord Perikarya of the Motor neuron degeneration (Mnd) mouse.

L. Callahan and J.E. Mazurkiewicz, Anatomy, Cell Biology, Neurobiology, Albany Med. Col., Albany, NY 12208

We previously reported that neurofilaments were abnormally distributed in spinal cord perikarya of the Motor neuron degeneration (Mnd) mouse, an animal model for human motoneuron disease. The study reported here investigated the distribution of tubulin, another major cytoskeletal element in neurons. An immunoperoxidase technique using antibodies to polymeric or dimeric tubulin was employed to analyze lumbar spinal cord perikarya in transverse and horizontal 30um vibratome sections. One severe and two moderate stage Mnd and two age-matched controls were examined.

In control perikarya, immunoreaction product for tubulin was distributed throughout the cytoplasm. Immunoreactivity of the peripheral cytoplasm was increased relative to the inner cytoplasm. In contrast, perikarya of Mnd contained prominent cytoplasmic regions devoid of tubulin immunoreactivity. This distribution was similar to that found for neurofilaments in affected cells in Mnd. The affected neurons were found predominantly in one lamina, suggesting a selection of neurons may be occurring in this disease. The lamina containing most of the affected neurons was lamina IX; the region containing alpha motoneurons. The alteration in the distribution of tubulin and neurofilament proteins suggests that changes in cytoskeletal function, including axonal transport, may play a role in the pathogenesis of the disease.

(Supported by NIH NS24426 and the ALS Association)

554.7

BRAIN UPTAKE AND DISTRIBUTION KINETICS OF BETA-N-METHYL AMINO-L-ALANINE IN THE RAT. Q.R. Smith, P. Pearson*1, N. Villacreses*, L. Wyatt*1, S. Markey2, I. Kopinl, and M. Duncanl. Lab. of Neurosciences, NIA; 1Intramural Res. Program, NINDS; and 2SAB, NIMH, NIH, Bethesda, MD 20892.

Beta-N-methylamino-L-alanine (BMAA) is a neurotoxic nonprotein amino acid present in cycads that has been implicated in the pathogenesis of the amyotrophic lateral sclerosis-parkinsonism dementia complex of the western Pacific. To evaluate its kinetics and brain uptake, BMAA (25-400 mg/kg) was administered to rats either acutely or chronically, and then plasma and brain concentrations were determined at various times thereafter by GC-MS. Following single dose i.v. injection, BMAA was cleared from plasma in a rapid distribution phase (Vd = 21.5 L/kg) followed by a slower elimination phase (t1/2 = 1 day). Brain uptake proceeded at a low rate (PA = 2.5 $\pm$ 0.2 x 10^{-3} ml/s/g) mediated in part by the large neutral amino acid carrier of the blood-brain barrier. Brain levels peaked within 8 hr after injection and then declined with a t1/2 similar to that of plasma. After two weeks of continuous infusion (100 mg/kg/day), steady-state brain concentrations equalled 10-30 ug/g and only moderately exceeded those in plasma. The results suggest that BMAA may reach potentially toxic levels in brain (>250 uM) following large doses (100-400 mg/kg). However, such doses are orders of magnitude greater than those available from diet or medical use of cycads.

554.8

IMMUNOHISTOCHEMICAL AND BIOCHEMICAL STUDIES ON WORSTER-DROUGHT DISEASE. D. Walker, H. Akiyama, T. Kawamata*, T. Pearson* and P.L. McGeer. Kinsmen Lab of Neurological Research, Univ. of British Columbia, Vancouver, B.C., Canada V6T 1W5

Worster-Drought and colleagues (J. Neurol Psychopath 14:27, 1933) described an autosomal dominant dementia with amyloid angiopathy and cerebral amyloid plaques. The index family has been followed through six generations (Plant et al. Brain:113, 1990). We have confirmed the original pathological findings in a 64-year old 5th generation decendent. There was extensive vascular amyloidosis and plaque formation particlarly in the inferior olive, hippocampus and amygdaloid nucleus, ischemic damage to selected areas, and patchy accumulations of neurofibrillary tangles. The amyloid deposits stained positively for amyloid P, but negatively for b-amyloid protein (antibodies courtesy D. Selkoe and T. Ishii). They also stained negatively for the prion protein (antibodies courtesy H. Wisniewski). These results ruled out Alzheimer and Gerstmann-Straussler as the cause of this disorder. The neurofibrillary tangles stained positvely for ubiquitin (antibody courtesy R. Kascsak), Alz-50 (antibody courtesy P Davies) and Tau-2. Partial sequence analyses of the transthyretin gene, using PCR amplified DNA extracted from the patient's brain, as well as gas-sequence analysis of transthyretin protein purified from the serum of an apparently affected daughter, showed no abnormality. The disease is unlikely to be a form of familial amyloidotic polyneuropathy. It appears to be a unique form of amyloidosis exclusively affecting brain tissue.

554.9

LOCALIZATION OF IL-1α AND IL-1β IN DISEASES WITH GLIOSIS, DEMENTIA, AND IMMUNE SUPPRESSION. L.C.Stanley and W.S.T.Griffin, Departments of Anatomy and Pediatrics, UAMS, Little Rock, AR 72205

Temporal lobe gliosis and immune suppression are present in several diseases having the clinical presentation of dementia. One manifestation of the immune suppression is a decrease in macrophage-derived IL-1 peripherally. In contrast, we have shown that IL-1 is elevated in reactive microglia and astrocytes in postmortem brain from individuals with Alzheimer disease (AD), Down syndrome (DS), and AIDS. Two forms of IL-1 have been described: IL-1α and IL-1β. We examined formalin-fixed, paraffin-embedded temporal lobe sections from AD, DS, AIDS, and controls, using specific antibodies to IL-1α and IL-1β. Cells containing IL-1α immunoreactive product were identified as either reactive microglia (process-bearing), brain macrophages (round), or endothelial cells. Microglia-like cells were found throughout grey and white matter; whereas, brain macrophages were located near blood vessels or within the choroid plexus. IL-1β immunoreactive product was found in endothelial cells and reactive astrocytes located near blood vessels. IL-1 within the lumen of blood vessels was predominantly labeled with the specific antibody for IL-1β. We conclude that excessive expression of IL-1α, by microglia and brain macrophages, and IL-1β, either produced or taken-up from the blood by astrocytes, accounts for the elevation of brain IL-1 in the diseases studied.

554.10

PROTEIN KINASE C ACTIVITY IN CEREBRAL CORTEX OF CATS WITH GM_1 GANGLIOSIDOSIS. G. Shanker, H. J. Baker* and A. L. Perrow* Department of Comparative Medicine, Bowman Gray School of Medicine, Winston-Salem, NC 27103.

GM_1 gangliosidosis is a lysosomal disease due to reduced activity of β-galactosidase with resulting decreased hydrolysis and lysosomal accumulation of glycolipids, glycoproteins, and other metabolites in brain, liver and other tissues. Pathogenesis of neuronal dysfunction in this and other lysosomal diseases is not understood. Our previous studies using feline models of the gangliosidoses indicate defective transmembrane signal transduction, including calcium dyshomeostasis. In vitro studies suggest that protein kinase C (PKC) inhibition by sphingolipids may play a role in these diseases. We measured calcium dependent PKC activity in cerebral cortex of cats with GM_1 gangliosidosis, age matched normal siblings, and unrelated normal controls using the Amersham assay procedure. Tissue preparations included: whole brain homogenate, P_2 fraction, synaptosomes and cytosol. PKC activity in unrelated normal cat brain was: homogenate 808 ± 55 pmoles/min/mg protein ($\bar{x}$ ± 1sd); cytosol 731 ± 63; P_2 284 ± 30; and synaptosomes 81 ± 6.4. Enzyme activity in all fractions was stable at - 70° for at least 10 days of storage. PKC activity in whole brain homogenate of 4 feline GM_1 gangliosidosis mutant cats was 1437 ± 90 pmoles/min/mg protein ($\bar{x}$ ± 1 sd), which was not statistically different from normal cat brain activity of 1166 ± 135. Further studies in progress will compare PKC activity in subcellular fractions of mutant and control cats. Supported by NINCDS grant NS10967.

554.11

^{3}H-PRAZOSIN BINDING IS REDUCED IN BRAIN OF PATIENTS WITH NARCOLEPSY. L.M. Dixon, M. Mamelak*, O. Hornykiewicz and S.J. Kish. Clarke Institute of Psychiatry, and Sunnybrook Hospital, Toronto, Canada.

Narcolepsy is a sleep disorder characterized by sleep attacks, cataplexy, sleep paralysis and hypnagogic hallucinations. Studies in human narcolepsy and its animal (canine) model have demonstrated that treatment with prazosin, an alpha-1-adrenergic receptor antagonist increases cataplexy and sleep time. We measured ^{3}H-prazosin binding (0.2nM) as well as levels of noradrenaline and its metabolite MHPG in four brain areas in autopsied brain of three narcoleptic patients and five age-matched controls. Binding was significantly decreased in the frontal cortex (-53%, $p<0.02$) and amygdala (-46%, $p<0.05$) but was normal in caudate and putamen. Although noradrenaline levels were normal in all brain areas studied, in frontal cortex MHPG levels (+131%, $p<0.01$) and turnover ratio MHPG/noradrenaline (+79%, $p<0.05$) were elevated, suggesting increased activity of locus caeruleus noradrenergic neurones and consequent down regulation of the post synaptic alpha-1-receptor. We suggest that altered brain noradrenergic mechanisms may be involved in the pathophysiology of human narcolepsy. (Supported by the American Narcolepsy Association).

554.12

IN SITU HYBRIDISATION STUDIES OF SYMPATHETIC GANGLIA IN MULTIPLE SYSTEM ATROPHY AND PURE AUTONOMIC FAILURE

OJF Foster and SL Lightman. Medical Unit, Westminster Hospital, 17 Page St, London SW1P 2AP U.K.

Although sympathetic ganglion cell numbers are reduced in Pure Autonomic Failure (PAF) but seem to be maintained in Multiple System Atrophy (MSA) (Matthews 1988), we know little about the behaviour of the residual ganglion cells in either disorder. Messenger RNA (mRNA) is relatively stable post-mortem and we have been able to apply in situ hybridisation histochemistry to the study of cell function in human sympathetic ganglia in PAF and MSA.

Eight micron frozen sections of sympathetic ganglia from MSA, PAF and control subjects were studied using probes directed against mRNA encoding tyrosine hydroxylase (TH), neuropeptide Y (NPY), the structural protein beta-tubulin (BT). Preliminary studies show reduced TH probe binding in the remaining ganglion cells in the PAF tissue studied, but well maintained levels in cells from MSA ganglia, with similar changes in NPY and BT probe binding.

Our initial studies suggest that ganglion cell biosynthesis of neurotransmitter and microtubular proteins is maintained in MSA but may be greatly reduced in the residual ganglion cells in PAF. Further work is underway to quantify these changes.

1) Matthews MR (1988) In: Autonomic Failure (Bannister ed). Chapter 29 pp 521-544. Oxford University Press.

554.13

THE G-PROTEIN SYSTEM IS ALTERED IN DIABETIC ENCEPHALOPATHY. A.M.Di Giulio, M.L.Malosio*, B.Tenconi*, M.L.Donadoni*, M.P.Abbracchio, F.Cattabeni and A.Gorio.Dept. of Med.Pharmacol.; Inst. of Pharmacol. Sci.; Univ. of Milano, Italy.

We have recently reported that the G-protein system is functionally altered in some CNS areas of diabetic rats. Two different stages of alloxan-induced diabetes were considered. In the early stage (5 weeks after alloxan injection) the G-protein mediated transductional signal was apparently unaffected troughout the CNS except for the retina, where a functional reduction of the Gi/Go system was observed. At later stages (14-16 weeks after alloxan injection) an involvment of the Gs retinal system as well as a reduced functional capacity of the striatal Gi/Go system were observed. The mRNA Northern Blot analysis revealed that at the earlier stage of diabetes the synthesis of Gi and Gs proteins is unaffected troughout the CNS except for the lumbar spinal cord. However, at a later stage of the disease the Gi mRNA is increased in retina and reduced in caudate. These data indicate that the various CNS areas are subjected to differential insults , thus suggesting a foundamental difference between the diabetic encephalopathy and the degenerative nature of the peripheral diabetic neuropathy.

555.1

GAP-43 mRNA LEVELS IN ASSOCIATION CORTEX DURING NORMAL AGING AND ALZHEIMER'S DISEASE. P. D. Coleman, A. B. Wadhams*, K. E. Rogers. Dept. of Neurobiology and Anatomy, University of Rochester, Rochester, N. Y. 14642

GAP-43 is a protein which has been found to be abundant in developing neurons as well as regenerating neurons. We have suggested that GAP-43 may be used as a marker of neuronal plasticity in aging brain, and conversely, Alzheimer's Disease(AD) could represent a loss of neuronal plasticity. Thus, mRNA levels of GAP-43 may reflect the degree of neuronal plasticity. RNA from frontal association cortex of AD patients and age-matched controls was isolated and checked for integrity by Northern blot analysis. Four serial dilutions of each sample were bound to a nylon membrane and hybridized under saturating conditions to a probe representing the entire coding region of rat GAP-43. Results showed no difference between AD and control samples in total yield of mRNA per gram of tissue obtained from each case. In normal aging, GAP-43 message levels decreased by approximately 47% between 50 and 70 years of age and did not significantly decline thereafter. GAP-43 message levels in AD samples were not significantly different from the levels found in normal age-matched controls. (Supported by AG 09016, AG 01121, AG 03644, AG 00107, PRG-89-120)

555.2

INCREASED SPECTRIN BREAKDOWN IN FIBROBLASTS FROM AGED AND ALZHEIMER DONORS. C. Peterson[1], P. Vanderklish[2*], P. Seubert[2], C. Cotman[1] and G Lynch[1,2]. [1]Dept. Psychobiology & [2]Bonney Center of Learning and Memory, University of California, Irvine, CA 92717, USA.

Several lines of evidence suggest that calcium homeostasis is altered by aging and Alzheimer's disease. For example it has been previously shown that there are deficits in calcium uptake and cytosolic free calcium but increases in bound calcium in fibroblasts from aged and Alzheimer donors (Ann. N.Y. Acad. Sci. 568: 262). Activation of a calcium sensitive protease, as evidenced by increased spectrin breakdown, occurs early in the course of neuropathology in several animal models. Since changes in calcium homeostasis may lead to abnormal regulation of calcium dependent proteases (e.g., calpain), the present study describes spectrin proteolysis in cultured skin fibroblasts from young, aged and Alzheimer donors. Cultured skin fibroblasts from young (21±1.2 yrs), aged (61±1.3 yrs) and Alzheimer (62.3±1.2 yrs) donors were obtained from the NIA Aging Cell Repository. Cells that had been deprived of serum for twenty-four hours were reexposed to 1% serum for 10 min. Electrophoretically separated proteins were transferred to nitrocellulose paper and spectrin breakdown products were immunodetected. The concentration of the 150 and 155 kD spectrin breakdown products was greatest in fibroblasts from Alzheimer donors (231.7±17.4%) and less in aged controls (183.9±15.6%) as compared to young donors (100±8.3%). Changes in calpastatin activities could not account for these alterations. Thus, abnormal calcium mediated proteolysis may contribute to the altered cytoskeleton dynamics that occur during aging and Alzheimer's disease. Supported in part by AG07855.

555.3

DISTRIBUTION OF PROTEASE NEXIN-1 (PN-1) mRNA IN RAT BRAIN AND ITS RESPONSE TO LESION . AN IN SITU HYBRIDIZATION STUDY. F. Gómez-Pinilla, S. Wagner, D. Cunningham and C. W. Cotman. Depts of Psychobiology and Genetics, University of California, Irvine CA 92717.

Protease nexin-1 is a serine protease inhibitor which shows neurite outgrowth activity *in vitro*. Recent evidence suggest that PN-1 may be involved in the etiology of Alzheimer's disease (AD) since PN-1 protein is reduced in AD brains. Thus, AD appears to be associated with an imbalance of proteases and protease inhibitors, possibly in part due to a break down of the blood brain barrier. In this study we have studied the cellular origin of PN-1 and the effect of injury on the expression of PN-1 mRNA in the hippocampus and cortical areas severely impacted by AD. We used a ^{35}S-labeled 603-base anti-sense RNA probe for *in situ* hybridization. The hippocampus was deafferented either by transecting the fimbria-fornix or by electrolitically lesioning the entorhinal cortex; rats were sacrificed at 1, 2, 3, 4, 5, 10, 14 or 30 days after lesion. Experimental and normal brains were processed for *in situ* hybridization. PN-1 mRNA hybridization was observed to be enriched in the hippocampus (dentate and pyramidal cell layer), cerebral cortex layer III, olfactory bulb periglomerular area. After either lesion, there was a transient increase (days 1 through 5) in PN-1 mRNA labelling density in the hippocampus ipsilateral to the lesion. There was not an exact topographical correlation between deafferented areas and lesion effect. This suggests that PN-1 can be induced as a general response to trauma as a possible mechanism to protect the brain and mobilize growth related events.

555.4

QUANTITATIVE IMMUNOREACTIVITY OF ALZHEIMER-LIKE ANTIGENS INCREASED BY ELDERLY HUMAN SERUM TREATMENT OF CULTURED NEURONS, G.J. Brewer, B.K. Miksanek* and J.W. Ashford, Southern Illinois Univ. Sch. Med. Springfield, IL 62794.

The mechanism for promoting the development of distinct types of lesions in the Alzheimer disease (AD) brain and other changes outside the brain is unknown. We examined unprotected neurons in culture to determine if exposure to serum would affect markers for Alzheimer brain lesions. Rat hippocampal neurons were first grown in a new serum-free culture medium, then exposed for 24 hr. to sera. Sera from AD patients and their spouses, but not young adult human or fetal bovine, each increased three molecular markers characteristic of Alzheimer senile plaques and neurofibrillary tangles: Alz-50, β-amyloid and MAP2. By quantitative immunofluorescence, neuronal exposure to the elderly human sera produced 2.5 to 3.8 fold increases in fluorescent area/cell and 17 to 37% increases in brightness of these three markers relative to no serum exposure. Anti-β-amyloid immunogold labeling and whole-mount electron microscopy revealed immunoreactivity associated with extracellular fibrillar deposits, adjacent to neurons in samples treated with elderly human serum which was reduced and restricted to apical dendrites in samples treated with young serum. These studies direct attention toward a common mechanism of induction of both plaques and tangles in AD. Supported by the Pearson Family Foundation and SIUSM.

555.5

INTRADENTATE COLCHICINE LESIONS: A PROPOSED ANIMAL MODEL OF WANDERING BEHAVIOR IN ALZHEIMER'S DISEASE. J.P. Ryan, M. Saxby*, T. McBain*, B. Kunkel*, D. Hoffman*, T. Brown*, and P. Stewart*. Dept. of Psychology, State University of New York at Plattsburgh, Plattsburgh, New York 12901.

Wandering behavior in Alzheimer's Disease is one of the most problematic characteristics of the disease. The human literature suggests that there may be two subtypes of wandering, goal-directed and non-goal-directed. The present study attempted to dissociate the two subtypes in an animal model using the Morris Water Maze. Twenty-nine Long-Evans hooded rats were divided into two groups. The experimental group (N=20) received 15 µg/uL of colchicine bilaterally into the dentate gyrus of the hippocampus **and the control group (N=9) received 1 µL of physiological saline. The animals were trained for six days (36 trials) in the water maze. The lesioned animals were significantly impaired in their ability to locate the goal in comparison to the control animals. Furthermore, the lesioned group itself exhibited marked differences in goal-finding behavior. The moderately impaired lesioned group failed to find the platform 40% of the time in comparison to the severely impaired group that failed to locate the platform 90% of the time. It is suggested that the behavioral impairment of the moderately impaired lesioned group mimics the spatial disorientation of the goal-directed wandering** observed in the Alzheimer patient. The hyperactive, motor-disinhibited behavior of the severely impaired group mimics the nongoal-directed wanderer.

555.6

CYTOCHROME OXIDASE INHIBITION IMPAIRS LEARNING AND HIPPOCAMPAL PLASTICITY: IMPLICATIONS FOR ALZHEIMER'S DISEASE. M.C. Bennett, D.M. Diamond, S.L. Stryker*, J.K. Parks* and W.P. Parker. Departments of Neurology and Pharmacology, UCHSC & VAMC, Denver, CO 80262

In recent work, Parker et al., (*Neurol.*, in press) reported a selective deficiency in the respiratory enzyme cytochrome oxidase (Complex IV) in blood platelets of Alzheimer's (AD) patients. This finding raises the possibility that a defect in the electron transport chain may play a role in the etiology of AD. Therefore, we tested the hypothesis that selective depletion of cytochrome oxidase impairs learning and physiological plasticity.

Adult male rats were each implanted with an Alzet minipump (2ML4) containing saline (CONTROL) or 160 mg/ml azide (AZIDE). On a shuttle avoidance task, AZIDE rats implanted 6-9 days prior to training did not show a learning curve and had longer escape latencies than did CONTROLS. This learning deficit could not be attributed to a sensory or a motor impairment. Further, in an 8-arm radial maze task, long-term (7 day) but not short-term (2 day) AZIDE treatment slowed the rate of acquisition. Finally, AZIDE rats were significantly impaired in their capacity to develop hippocampal LTP.

These data indicate that chronic, selective depletion of cytochrome oxidase by azide produces deficits in learning and hippocampal plasticity. We suggest that the azide-induced inhibition of cytochrome oxidase in rats constitutes a novel animal model of AD.

555.7

ALUMINUM-INDUCED NEURITE OUTGROWTH IN CULTURED HIPPOCAMPAL NEURONS RESEMBLES NEURITE SPROUTING SEEN IN AGING AND ALZHEIMER'S DISEASE. E. Uemura and R.K. Lartius. Department of Veterinary Anatomy, Iowa State University, Ames, IA 50011.

It has been shown that the aluminum content in the human brain increases with age (up to 25 μM), and it is particularly high in those with Alzheimer's disease (up to 910 μM). we found that 100 μM aluminum in culture media can induce extensive neurite outgrowth (i.e., elongation and branching of neurites) and sprouting (i.e., outgrowth of filiform-like processes from neurite varicosities) in some hippocampal neurons derived from brazilian short-tailed opossum. Such neurite changes occured within 24 hours of aluminum exposure. Neurites exposed to aluminum appeared to grow with no clear direction. Further, there was no statistical difference in neurite length between neurons exposed to aluminum for 24 hours versus four days. Some neurons also displayed sprouting of neurites. Such sprouting always originated from a globular enlargement at the focal area of the neurite along the neurite shaft and at terminal end of the neurite. It appears that brief exposure to low level aluminum is sufficient to promote extensive neurite elongation and sprouting in some hippocampal neurons.

555.8

PERSISTENT CHOLINERGIC INNERVATION OF CEREBRAL CORTEX DESPITE DEPLETION OF CORTICAL NEURONS. S.L. Minger & P. Davies*.
Dept. of Pathology, Albert Einstein College of Medicine, Bronx, NY 10461

A controversial theory in Alzheimer's disease research posits that degeneration of the basal forebrain cholinergic nuclei is due to the loss of trophic factors secondary to a reduction in the number of cortical neurons. We have used the in utero application of methylazoxymethanol acetate (MAM) to directly probe this relationship. MAM (15mg/kg,i.p.) or 0.9% NaCl (1.0ml/kg) was administered to pregnant Sprague-Dawley rats on gestational days 14+15. Brains of the offspring of MAM-treated and control animals were utilized for morphological analysis at 2 months of age, and for neurochemical studies at 2 and 6 months. Anatomically matched sections through the anterior to posterior extent of the brains were rigorously analyzed. The MAM-treated animals demonstrated a 25-35% reduction in cortical cross-sectional area, a 40-65% reduction in cortical volume, and a 50-70% reduction in total cortical neurons in layers II/III, as compared to age and sex matched controls (all values $p<.001$). In contrast to this extensive cortical lesion, in the 2 month old MAM-treated rats cholinergic markers (ChAT and AchE) were significantly increased in each of the four neocortical regions analyzed. The increases in ChAT levels ranged from 25% to 56% in the neocortex, with significant increases also found in the striatum and hippocampus. Furthermore, this relative, global cholinergic hyperinnervation of the MAM-treated brain was found to be sustained at 6 months of age in all regions examined. It will be of interest to determine if the hypocellular cortex can maintain a cholinergic hyperinnervation over the life span of the MAM-treated rat.

555.9

ORGANIZATION OF NEUROPEPTIDERGIC SYSTEMS AFTER CHOLINERGIC LESIONS OF THE NUCLEUS BASALIS IN RATS. J.W. Unger and W. Lange*. Department of Anatomy, University of Munich, FRG.

Despite the presence of a number of neurochemical alterations, the cholinergic deficit is a consistent finding in brains of patients with Alzheimer's disease (AD). Since previous studies have shown quantitative changes of cortical somatostatin concentrations in AD patients, as well as rats after lesions of the basal forebrain, our investigation evaluates the response of peptidergic networks after cholinergic deafferentation of several brain regions. 4 weeks and 3 months after bilateral lesions of the nucleus basalis, cell sizes, morphology and density of fibers or terminals are investigated by immunohistochemistry. The distribution of somatostatin and neuropeptide Y neurons in cortex and amygdala remains unchanged after 4 weeks; only minor atrophy of these cells is seen. In addition, galanin fibers in the basal forebrain are spared in lesioned animals. So far, our findings support the hypothesis that cholinergic deafferentation may present only part of the factors that cause widespread neurochemical changes in AD.
(Supported by DFG grant Un 59/2-1)

555.10

MOUSE TRISOMY 16 NEURONS MAINTAINED BY NEURAL TRANSPLANTATION: A POSSIBLE MODEL OF NEUROPATHOLOGY IN ALZHEIMER'S DISEASE. B. Ault, A. Fine & S.I. Rapoport. Lab. Neurosciences, NIH, Bethesda, MD 20892 and Dept. Physiology & Biophysics, Dalhousie Univ., Canada B3H 4H7.

Because of homology between regions of mouse chromosome 16 and human chromosome 21, the trisomy 16 mouse is considered a model of human trisomy 21 (Down syndrome). All trisomy 21 individuals develop neuropathology indistinguishable from Alzheimer's disease after the third decade of life. It would therefore be of great interest to examine trisomy 16 tissue at various periods of survival for any markers of Alzheimer's-like neurodegeneration. However, trisomy 16 fetuses die in utero or shortly after birth. We have attempted to maintain trisomy 16 and control tissue by the use of neural grafting techniques.
Hippocampal and neocortical areas were dissected from brains of trisomy 16 and littermate control fetuses after 14-15 days gestation. After incubation with 0.05% trypsin, the tissue was dissociated in a minimum volume of medium. Viability was better than 90% as assessed by ethidium bromide exclusion. 1 uL of tissue suspension was ejected into the right striatum of young adult sibling hosts. Operated mice were perfused after 2 weeks to 6 months survival. 7 mice with trisomy 16 grafts and 8 mice with control grafts were examined. Cresyl violet staining showed surviving grafted tissue in all animals except one (trisomy 16 graft; 3 week survival) which was well integrated with the host brain. Trisomy 16 grafts were smaller than corresponding control grafts on average, but no obvious neuropathology was observed. However, in trisomy 16 grafts at 4 and 6 month survival times selective expression of some Alzheimer's-associated markers was noted.

555.11

GRAFTING OF FETAL HIPPOCAMPUS IN A RODENT EXCITOTOXIC MODEL OF ALZHEIMER'S DISEASE. D.A.Turner, W.-Q.Dong and D.L.Deupree. Neurosurgery, Duke Univ. Med. Ctr. and Research Service, Durham VAMC, Durham, NC, 27710.

Since many of the early pathological and behavioral changes associated with Alzheimer's disease may be linked to hippocampal damage, we have developed an excitotoxic lesion of bilateral hippocampal dysfunction as a model. Lesion induction involved a slow infusion of N-methyl-D-aspartate (NMDA) into the lateral cerebral ventricles over 2 weeks, to a total dose of 1 mg NMDA. The animals were behaviorally analyzed using a water maze test (using an index of cumulative escape time - CEM) and then underwent neural grafting of 17-18 day fetal hippocampus as chunks into the lateral ventricles. At time points after the grafting procedure the animals were again behaviorally tested, and physiological recordings were performed *in vitro* on the grafts, to assess synaptic integration of the tissue. The goal of the grafts as a treatment paradigm was to partially reconstruct the CA1 circuitry.

Histological analysis of the lesion showed bilateral CA1 cell loss, with moderate accompanying dentate granule damage. Behavioral analysis at the 1 mg NMDA dose showed a subset of lesioned animals with behavioral abnormalities (n=13/24; CEM = 1784 ± 307 s; mean ± SD), compared to control animals (n = 12; CEM = 1003 ± 405 s; $P < 0.05$ by t-test). At one year this behavioral abnormality was persistent in a subset of animals (n= 7/13; CEM = 1764 ± 294 s). The effects of the grafts on the behavioral abnormality were mixed, with a subset showing an improvement (n=6/10; CEM = 650 ± 217 s; $P < 0.01$ by t-test). Physiological recordings from the grafts in slices showed some synaptic connectivity to the host, but the grafts were in general not well integrated into the host hippocampus.

Thus, the integration of the grafts into the host hippocampus and behavioral effects were only moderate. Further characterization will include long-term anatomical and behavioral analysis and improved graft integration with suspension grafts. This model may have considerable relevance for the analysis of fetal grafting paradigms in Alzheimer's disease. Supported by grants from ADRDA, VAMC and B.S. Turner.

556.1

SACCADIC EYE MOVEMENTS IN SCHIZOPHRENIA: EVIDENCE FOR IMPAIRED SENSORIMOTOR GATING OF INTERNAL REPRESENTATIONS. D. Hommer, A. Radant* and S. Nickoloff*. GRECC, VAMC, Seattle, WA 98108.

To investigate the proposal that schizophrenia is a disorder affecting the utilization of representational knowledge, we used infra-red oculography to measure both visually and internally guided saccades during a task in which, initially, the target stepped 20° between two points at unpredictable intervals. Next, it moved in a completely predictable pattern. Finally, the target resumed the initial pattern of predictable location but unpredictable timing. Horizontal eye movements were recorded from 26 neuroleptic-treated and 8 drug-free schizophrenics, 25 normals and 8 patients with obsessive compulsive disorder. Latency and accuracy of visually-guided saccades and the frequency of adaptive predictive saccades did not differ among the groups. However, when the target motion switched from predictable to unpredictable schizophrenics made significantly more premature saccades directed towards the next target. The frequency of these maladaptive anticipatory saccades significantly correlated with thought disorder. These saccades may result from a failure in the inhibitory gating of premotor commands derived from cortical representations of target motion.

556.2

IMPLICIT MEMORY IN SCHIZOPHRENIA. B.L. Schwartz, R.B. Rosse*, and S.I. Deutsch. Psychiatry, VA Medical Center, Washington DC, 20422.

Schizophrenic patients are impaired on explicit recall tests, which depend on higher-order or conceptual cognitive processes. The question in this research was whether schizophrenic patients would be impaired on an implicit memory test that also depends on conceptual processes. We examined this issue in two experiments. In Experiment 1, we replicated the deficit in recall in schizophrenic patients. In Experiment 2, we examined implicit memory in schizophrenic patients using two tests, one that depended on conceptual processes (category production) and one that did not (word identification). The results of Experiment 2 showed that performance for schizophrenic patients did not differ from the performance of control subjects in either the category production or word identification tests. Implicit memory was unaffected in schizophrenia, irrespective of the nature of the cognitive processes involved in the test. These results suggest that conceptual processes in implicit memory tests may differ from those in explicit memory tests.

556.3

SCHIZOPHRENIA PSYCHOPATHOLOGY FOR THE NEUROSCIENTISTS. W.T. Carpenter, R.W. Buchanan* and B. Kirkpatrick. Maryland Psychiatric Research Center, P.O. Box 21247, Baltimore, MD 21228.

Current approaches to diagnosis, proven reliable and valid for many clinical and administrative purposes, are grossly inadequate for the neuroscience-based investigation of schizophrenia. Multiple criteria drawn from divergent aspects of behavior are poorly suited guides for developing animal models. An alternative approach to the use of diagnoses is to subdivide schizophrenia into discrete domains of psychopathology. Five such domains have been defined: expressive symptoms, cognitive symptoms, incongruity of affect, deficit functions, and neurologic manifestations. The neuroscience application of domains can be illustrated in two areas: 1) defining the phenotype for linkage studies; 2) establishing animal models for specific psychopathologic attributes. The authors believe that this shift in focus from diagnosis to domains will facilitate neuroscience investigations of schizophrenia by providing more relevant and applicable psychopathologic targets. This approach may diminish the false negative ascertainment in pedigree-based studies and decrease the speculative inference of animal models to schizophrenia. An animal model of social affiliation, anhedonia, or attentional dysfunction will require less speculative inference, and the relevance to schizophrenia will be more straightforward.

556.4

CORTICAL FOLDING IN THE TEMPORAL LOBE OF SCHIZOPHRENICS. E. Armstrong. Yakovlev Collection, Amer. Regis. Pathol., A.F.I.P., Washington, D.C. 20306.

The degree of cortical folding, as measured by the gyrification index (GI), was used to test the hypothesis that sulcal/gyral patterns in the temporal lobe differed between schizophrenic and normal brains. The GI was measured separately for the pole, dorsal, medial and lateral regions of the temporal lobe of 10 normal, 7 schizophrenic brains with leukotomies and 6 nonschizophrenic brains with leukotomies. The brains come from the Yakovlev Collection.

Both sets of leukotomized brains had a decrease in folding in the dorsal (Sylvian) region compared to normals, but an increase in relative amount of folding in the left medial cortex at the level of the anterior hippocampus. The GI's in the schizophrenic brains differed from the others in having a decreased degree of folding in the left medial cortex at the level of the amygdala, suggesting specific anomalies in this region. The medial cortex is from the amygdala to the medial bank of the occipito-temporal sulcus and includes much of the entorhinal cortex.

Supported by NIMH 45594.

556.5

AGE-RELATED CT MEASURES IN SUB-GROUPS OF SCHIZOPHRENIC PATIENTS. Arthur Kling, Alan Steinberg, Peter Lucas, Neena Sachinvala, Ken Tachiki, Harald von Scotti, R. F. Ritzmann. Sepulveda VAMC, UCLA, Los Angeles, CA 91343.

Seventy-two schizophrenic patients and 41 controls had head CT scans. Patients were subtyped according to DSM III-R criteria. Measures were made of the area of the lateral ventricles and the amount of cortical atrophy in various brain regions. VBR differentiates between paranoid and residual/undifferentiated schizophrenic subgroups between the ages of 30-49. Total brain atrophy differentiates residual/undifferentiated schizophrenics from paranoid schizophrenics and/or controls after the age of 50. After the age of 40, atrophy in the sylvian region differentiates between schizophrenics and controls, regardless of sub-classification. To assess the possibility that alternative diagnostic classification systems would yield more robust CT differences, a subgroup of patients was classified according to three alternative typologies: Positive/Negative; Type I/Type II; Deficit/Nondeficit. No significant regrouping of patients emerged. Therefore, no change in CT findings was generated via these alternative classification methods. Supported by the Research Service, Sepulveda VAMC.

556.6

REGIONAL VARIATION OF NEUROANATOMICAL BRAIN ABNORMALITIES IN SCHIZOPHRENIA M. S. Myslobodsky, R. Coppola, E. F. Torrey*, D. R. Weinberger, NIMH Neuroscience Center, St. Elizabeths, Washington, DC 20032

Structural brain pathology encountered in schizophrenic patients poses a question whether the abnormalities are centered predominately at the limbic-prefrontal system. We answered this question by comparing the size of the septum with the size of the cuneus and the sucal pattern of the occipital lobe. The latter remains a unique brain province in that it has not been implicated in schizophrenia. Subjects were 19 pairs of monozygotic twins discordant for schizophrenia (range of discordance period was 4-24 yr.). For all pairs MR images (1.5 Tesla GE scanner) were obtained using identical T1 weighted spin echo pulse sequences (TR/TE=800/20). Sagittal cuts (5 mm thick) were analyzed to compare the size of the septum, cuneus, and the course of the medial occipital sulci. The 'variance zone' for the parieto-occipital and retrocalcarine sulci, the slopes of the right and left retrocalcarine sulci, and the the size of the cunei were practically identical between hemispheres and between the two groups. There was a small (11.4%) increase of the size of the septum in the impaired group. Two-sample sign test confirmed a high significance of this difference (p=.002). Thus, the impaired twin could be differentiated by increased septal area, but not by the anatomy of the occipital lobes within the same geometrical space of the brain.

556.7

EVIDENCE FOR DIFFUSE GRAY MATTER ABNORMALITIES IN SCHIZOPHRENIA R.B. Zipursky*, K.O. Lim*, A. Pfefferbaum Department of Psychiatry and Behavioral Sciences, Stanford University School of Medicine and VA Medical Center, Palo Alto, CA 94304.

A Magnetic Resonance Imaging (MRI) study was undertaken to determine the extent to which the greater ventricular and sulcal volume found in schizophrenic patients is due to differences in gray matter versus white matter volume.

Axial MRI scans were obtained using a GE Signa 1.5 T scanner from 22 patients meeting DSM-III-R criteria for schizophrenia and 20 healthy community volunteers. All subjects were right-handed male veterans, 20 to 45 years of age. Cerebrospinal fluid volume, gray matter volume and white matter volume were determined for eight 5mm thick sections per subject using a modification of the semiautomated approach described by Lim and Pfefferbaum (J Comput Assist Tomogr, Vol.13, No.4, 1989). Cortical areas were subdivided into multiple subregions in order to determine whether differences could be localized.

Within the cerebral cortex of the community volunteers, percentage gray matter volume but not percentage white matter volume was significantly correlated with age ($r = -.76$, $P < 0.001$). After correcting for the effect of age, the schizophrenic group was found to have significantly lower cortical gray matter volume than the control group (mean $Z = -1.88$, $P < 0.001$). Significant differences in percentage gray matter were found in all regions. No significant differences were found in percentage white matter in the cortex as a whole or in any of the cortical subregions.

These results are consistent with the view that relative gray matter volume is abnormally low throughout the brain in patients with schizophrenia as a group. It remains to be established whether specific regions are disproportionately affected.

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556.8

GROWTH PROMOTING AGENT FOUND IN SCHIZOPHRENIC CSF CHANGES NEUROBLASTOMA CELL PROPERTIES. S.Shirabe*[1,2], J.P.Schwartz[2], and J.R.Stevens[1]. 1) NIMH, St. Elizabeths Hosp., Washington DC 20032, & 2) CNB, NINDS, NIH, Bethesda MD 20892.

This study was undertaken to look for a transmissible agent involved in the etiology of schizophrenia. The human neuroblastoma cell line SH-EP (NB) was incubated for 5 days with cerebrospinal fluid (CSF) from patients with schizophrenia (Sch) of 2-22 years duration (mean 8.9 yr) from St. Elizabeths Hosp., Washington DC, or various types of control CSF, after which cells were passaged at 2 week intervals. After 2-6 months culture, NB cells which had been treated with fresh CSF from 12/12 Sch patients showed 30-210% higher density growth than 15 control CSF-treated NB cell cultures ($p<.01$). The growth promotion could be transmitted by cell-free media from Sch CSF-treated cells. Seven of 9 Sch CSF- but only 1 of 13 control CSF-treated cultures showed increased size and/or number of colonies upon growth in soft agar. NB cells treated with Sch CSF showed changes in their intracellular neurofilament distribution by immunocytochemistry, using several different antibodies. Experiments are underway to identify the agent responsible for the cell transformation.

556.9

PARANOID SCHIZOPHRENIA: FREE AMINO ACIDS IN CEREBROSPINAL FLUID. E. Bonilla, D. Prieto-Rincón*, L. Piñerúa-Shuhaibar*, A.L.N. Prasad*, A. Arrieta*. Instituto de Investigaciones Clínicas, Universidad del Zulia and Inbiomed-Fundacite, Apartado 1151, Maracaibo, Venezuela.

Few publications have appeared regarding the levels of free amino acids in cerebrospinal fluid (CSF) of schizophrenic patients. We now report the results of the determination of 21 free amino acids levels in CSF of five non psychiatric controls (3 women, 2 men; mean age $\pm$ S.D., 41 ± 11.3 years) and seven paranoid schizophrenic patients (4 women, 3 men; mean age, 26.7 ± 8.1).

The samples of CSF were taken between 8 and 11 a.m., after patients and controls had been at bed rest for at least 8 hours and fasting for 14 hours. Lumbar puncture was made between L4 and L5. The amino acids analysis was performed by HPLC. The schizophrenic patients had higher ($p < 0.05$) CSF concentrations of glutamine than controls (547.4 ± 81.4 and 354.5 ± 118.7 µmole/liter, respectively). The levels of the remaining 20 free amino acids did not differ from control values. No significant correlation was detected between age, sex, and content of amino acids in CSF.

We could not confirm the findings of Bjerkenstedt et al (Br.J.Psychiat. 147: 276, 1985) that CSF levels of histidine were elevated in schizophrenic patients. Similarly we did not find any significant increase in the concentrations of CSF alanine, glycine, leucine, and phenylalanine as reported by Reveley et al (Biol.Psychiat. 22: 413, 1987).

556.10

DIFFERENTIAL NEURAL CIRCUITS IN SCHIZOPHRENIA. R.W. Buchanan*, W.T. Carpenter, F. Wood, G. Thaker, C. Tamminga. Md Psych Res Ctr, Baltimore, MD 21228

Subclassification of schizophrenic symptoms leads to the identification of discrete neural circuits associated with specific domains of psychopathology. We have studied two of these domains, positive psychotic symptoms and primary, enduring negative or deficit symptoms for discriminating neuroanatomical, neuropsychological, and electrophysiological characteristics. Substantial evidence now exists to suggest that different neural circuits are involved in the production of these symptoms. Limbic circuits appear to mediate positive symptoms and distinguish schizophrenic patients from controls. In contrast, deficit is distinguished from non-deficit schizophrenia by significantly reduced regional glucose utilization (mg glucose/100 gm tissue/min) in the thalamus (deficit: 8.1 ± 0.8; non-deficit: 10.4 ± 0.7) and in multiple regions of the frontal and parietal cortices. Deficit patients also exhibit significantly increased impairment on neurological measures of parietal lobe function (deficit: 3.29 ± 1.10; non-deficit: 1.47 ± 1.12) and significantly increased volitional saccadic latency (secs) (deficit: 441 ± 94; non-deficit 351 ± 70), an eye-tracking measure sensitive to frontal and/or parietal lobe impairment. These data implicate the involvement of the thalamus and frontal and parietal cortices in the production of deficit symptoms.

556.11

PET/FDG LOCALIZATION OF METABOLIC CHANGES IN SCHIZOPHRENIA. G.K. Thaker, A. Summerfelt*, M.B. Yablonski*, T.N. Chase, C.A. Tamminga, Maryland Psychiatric Research Center, University of Maryland at Baltimore, Baltimore, MD 21228

Regional analysis of cerebral glucose utilization may serve to localize areas of psychopathology in schizophrenic brain. PET/FDG studies were carried out in drug-free schizophrenic patients to test the hypothesis that areas of altered metabolism would parallel those brain areas in animals affected by the psychotomimetic PCP. Twelve young schizophrenic subjects with mixed florid psychotic symptoms and matched normals were scanned using ^{18}F-2-deoxyglucose on the NeuroPET scanner; five transverse planes in each subject were analyzed blindly using ROIs matched to a human brain atlas. Results showed no differences between schizophrenics and normals in neocortical or extrapyramidal areas, but a significant decrease in glucose metabolism in limbic cortex in subjects with schizophrenia. Specifically, metabolic rates of patients were reduced in hippocampus and anterior cingulate. This distribution of metabolic changes parallels the metabolic alterations in rat brain following PCP and MK801. Differences in schizophrenic subgroups will be reported.

556.12

THE COADMINISTRATION OF AMPHETAMINE AND HALOPERIDOL IN SCHIZOPHRENIA. T. E. Goldberg*, L. B. Bigelow, D. G. Daniel, J. E. Kleinman*, D. R. Weinberger*. Clinical Brain Disorders Branch, NIMH Washington, D.C. 20032

In this study, we attempted to determine if an acute dose of dextroamphetamine might have positive effects on affect and cognition in schizophrenic patients maintained on haloperidol. We based this premise on the rationale that dopamine type I receptors in frontal cortex might be indirectly stimulated by amphetamine, resulting in improved affect and cognition, while the potential psychotogenic effects of amphetamine stimulation of subcortical type II receptor sites would be prevented by haloperidol blockade. In a double-blind placebo crossover study, 21 patients with chronic schizophrenia received a single oral dose of amphetamine at .25 mg/kg. All patients were receiving .4 mg/kg daily of haloperidol. Six patients were judged by clinical rating systems to have improved in terms of affect and engagement with the environment. Improvement was associated with enlarged cerebral ventricles and also with increases in blink rate from placebo to active drug condition. Amphetamine also improved some parameters of performance on the Wisconsin Card Sorting test of concept formation and set shifting, though it did not result in changes in tests of memory or attention. The possibility that a dopamine agonist in conjunction with a dopamine type II receptor antagonist might prove efficacious in schizophrenia may have implications for understanding the neural systems involved in this disease.

556.13

[^{3}H]PAROXETINE BINDING IN POSTMORTEM BRAINS OF SCHIZOPHRENICS, SUICIDES, COCAINE ADDICTS AND CONTROLS. M. Laruelle, M. F. Casanova*, D. R. Weinberger and J. E. Kleinman, CBDB, NIMH Neuroscience center, Washington D.C. 20032.

[^{3}H]Paroxetine is the ligand of choice to label the serotonergic uptake sites in human brain (Laruelle, M., Biol. Psychiatry, 24:299, 1988). Because of the implication of the serotonergic system in schizophrenia and suicide, we performed post mortem saturation analysis of [^{3}H]paroxetine (0.065 to 2nM) specific binding (defined as the binding displacable by 1µM citalopram) in the frontal pole of patients suffering from schizophrenia (n=10), chronic schizoaffecive illness (n=5), non psychotic suicide (n=8) and matched controls (n=10). Samples from cocaine addicts were also analysed (n=9), due to the reported toxicity of chronic cocaine administration for serotonin uptake sites in rats (Terry L.M., Soc. Neurosc. Abst., 322.11, 1989). Kd values did not exhibit significant differences between the groups. Bmax values were significantly differents (Anova,p<0.05): controls 84±13 fmol/mg of protein (mean±SEM), schizoaffectives 81±8 fmol/mgP, schizophrenics 48±5 fmol/mgP, suicides 48±2 fmol/mgP and cocaine addicts 64±10 fmol/mgP. Post-hoc analysis showed a significantly lower Bmax in schizophrenics and suicides compared to controls. These results suggest an abnormality of the serotonergic presynaptic system in schizophrenia and suicide but fail to support in human the notion of toxicity of cocaine toward these terminals as described in rats.

556.14

Abstract Title: A COMPARISON OF CHRONIC SCHIZOPHRENICS WITH INTERICTAL PSYCHOTICS

Author(s) Name: Estelle Toby Goldstein, M.D., Sheldon H. Preskorn, M.D., Beryl Silkey, ScM., Linda Haug

Patients with the interictal psychosis of partial complex epilepsy (n=18) were compared with patients with chronic schizophrenia (n=17). The patients had been followed in the inpatient and outpatient units of the Wichita Veterans Administration Medical Center Psychiatry Service. Epileptic patients did not significantly differ from schizophrenics in age at index hospitalization (Mean ± SD = 45.4 ± 9.4 yrs vs. 43.4 ± 10.8 yrs, respectively; t=0.58, df=33, N.S.). Schizophrenic patients were about ten years younger at age of DSM-IIIR diagnosis than the epileptic patients, and the latter had a broader range of ages at which diagnosis was made (Mean ± SD = 23.2 ± 6.6 yrs,; 32.5 ± 12.5 yrs, respectively, t=2.54, df=28, p<.01). Schizophrenic patients became psychotic at an earlier age than epileptic patients (Mean ± SD=22.6 ± 6.8 vs. 33.2 ± 10.8; Student's t=3.05, 27df, p<.005).

The epileptic group was more likely to have had a documented history of head injury (X^2=8.31, 3df,p<.05) than the schizophrenic group. Documentation of other major injuries, however, did not differ significantly between the two groups (X^2=.55, 3df, N.S.).

The medical histories of these two groups were not notably different. Two patients in the chronic schizophrenic group had a family history of schizophrenia (maternal side),compared to no family history in the epileptic group. Family history for non-psychotic psychiatric illness (alcohol abuse, depression, other) was equally represented in both groups (five in each group).

Additional comparisons of these syndromes are made, with special reference to clinical features of the psychosis, other behavior disturbances and treatment response.

556.15

ELEVATED D2 DOPAMINE RECEPTOR DENSITY IN 26 SCHIZOPHRENIC PATIENTS: L. Tune, D.F. Wong, H.N. Wagner, R.F. Dannals, The Johns Hopkins Hospital Medical Institutions, Baltimore, MD 21205

Utilizing 11-C-N-methylspiperone positron emission tomography (PET) striatal D2 dopamine receptor density (Bmax) was compared in 26 chronic schizophrenic subjects, 21 of whom were drug-naive at the time of scan (and an additional 5 with minimal neuroleptic exposure and 14 controls). All patients satisfied DSM-IIIR criteria for chronic schizophrenic illness. The average age was 37.65 +/- 3.5 years. The average duration of illness was 4.63 +/- .89 years (without prodrome) and 7.22 +/- 1.29 years with prodrome. Dopamine receptor density (Bmax) in schizophrenic subjects (32.35 +/- 17.89) was significantly elevated when compared to control subjects (15.46 +/- 9.26). Bmax overlapped with normal controls in 11 of 26 subjects. Clinical and neuropsychological test results were then compared with Bmax values and will be presented.

556.16

POSTMORTEM STUDY OF DOPAMINE AND ITS METABOLITES IN BILATERAL AMYGDALAS OF SCHIZOPHRENICS, COCAINE ADDICTS AND CONTROLS. B.S. Kolachana, M. Laruelle, M. F. Casanova* and J.E. Kleinman. NIMH Neuroscience Center at St Elizabeths, Washington D.C., 20032

Previous studies (Reynolds G.P., Nature, 305:527, 1983) have reported a higher concentration of dopamine (DA) and, to a lesser extent, homovanillic acid (HVA), in the left compared to right amygdalas of patients with schizophrenia. In an attempt to replicate these findings, we measured DA, HVA and DOPAC in the right and the left amygdalas of schizophrenics (n=4), cocaine addicts (n=5) and matched controls (n=6). For DA, results were (Means±SEM) as follows: controls: right 0.83±0.36ng/mg of protein, left 0.43±0.09ng/mgP; schizophrenics: right 0.66±0.37ng/mgP, left 1.46±0.94;ng/mgP; cocaine addicts: right 1.70±1.1ng/mgP, left 1.04±0.49ng/mgP. Neither the diagnosis factor, the hemispheric side factor, nor the diagnosis x hemispheric side interaction showed a significant effect when analysed with a 2 factor repeated measure Anova. Results of HVA and DOPAC levels were also negative. Nevertheless, the schizophrenic group was the only group that showed higher DA levels in the left side compared to the right. This was also true for HVA. More samples should be studied before a conclusion could be reached.

556.17

PCP-INDUCED DOPAMINE RELEASE IN VITRO: TACHYPHYLAXIS AND RECOVERY OF RESPONSE. L.P. Dwoskin, L.L. Leibee, S.T. Buxton and J.M. Carney. Div. Pharmacol. Exp. Ther., Col. Pharmacy and Dept. Pharmacol., Col. Med., Univ. Kentucky, Lexington, KY 40536.

Abuse of phencyclidine (PCP) produces behavioral effects resembling schizophrenia, which may be due to activation of CNS dopaminergic systems. Using HPLC-EC, endogenous dopamine (DA) and dihydroxyphenylacetic acid (DOPAC) were measured in superfusates from striatal slices taken from DBA/2J mice. Two slices were placed in each of two superfusion chambers and were superfused (1 ml/min) with Krebs' buffer for 60 min. Subsequently, chambers were superfused for 60 min with control buffer or with buffer containing PCP ($3x10^{-5}$M), followed by control buffer for a variable period (0, 30, 60 and 120 min) followed by a second, 60-min exposure to PCP ($3x10^{-4}$M). Initial exposure to PCP resulted in a peak increase in the concentration of DA and DOPAC (190 and 450 pg/ml/mg, respectively). Despite the continued presence of PCP, DA and DOPAC concentrations returned to basal levels, indicating rapid desensitization. Preexposure to PCP diminished the subsequent response to PCP (70% of control for DA, 40% for DOPAC). However, DA and DOPAC concentrations were not different from control when superfusion for 30 or 60 min period, respectively, was interposed between PCP exposures. Therefore, the diminshed response was not due to depletion of the releaseable pool of DA, but rather to a dynamic adaptive response of the dopaminergic neuron. Supported by NIMH MH42934, UKMC Fund and NIDA Contract 271-87-8133).

556.18

POSSIBLE MESOPONTINE CHOLINERGIC HYPERPLASIA IN SCHIZOPHRENIA. C. Karson, E. Garcia-Rill, J. Biedermann*, J. Smith*, R. Skinner, R. Mrak and M. Husain*. Dept. of Anatomy, Univ. of Arkansas for Med. Sci., Little Rock and V.A. Hospital, North Little Rock, AR.

Pedunculopontine nucleus (PPN) neurons, along with adjacent catecholaminergic nuclei, have been implicated in the modulation of sleep and movement. This cholinergic cell group appears to be the generator of the P1 auditory evoked potential (Buchwald et al., 1990) which in turn fails to habituate in schizophrenia (Freedman et al. 1983). Cholinomimetics produce sleep pattern and affective disturbances similar to schizophrenia and a role for cholinergic hyperactivity has been proposed in this disorder. Human brain tissue was obtained from four diagnosed schizophrenics (age 63±10) and from five control subjects (age 60±9). One half of the mesopontine brainstem was fixed and frozen sections processed for NADPH diaphorase histochemistry (which labels cholinergic mesopontine cells) and tyrosine hydroxylase immunocytochemistry (which labels catecholaminergic cells). The cell number and size of cholinergic PPN and laterodorsal tegmental (LDT) nuclei, as well as the nonadrenergic locus coeruleus (LC) were measured. Wilcoxon Test P<0.05 = ***

Cell Number	PPN	LC	LDT
Control	8,500±1621	11,353±4,791	6,268±3,526
Schizophrenia	18,275±8220***	11,496±2,956	12,266±7774
Cell Size (sq. um)	PPN	LC	LDT
Control	517±67	1032±105	625±37
Schizophrenia	517±44	761±106***	553±58

These preliminary findings suggest the presence of a greater number of cholinergic PPN neurons in the brains of schizophrenics. Such a hyperplasia appears consistent with the proposed fault in programmed cell death in this disease (Feinberg 1982), perhaps leading to cholinergic hyperactivity. The reduced cell size present in LC could be a result of neuroleptic treatment, and may contribute further to the disinhibition of PPN in this illness.

556.19

THREE BRAIN ANTIGENS IN SCHIZOPHRENIA

W.G. Honer, C.A. Kaufmann, J.E. Kleinman, M.F. Casanova*, J. Gleeson, P. Davies* Dept. of Pathology, Albert Einstein College of Medicine, Bronx, NY 10461

We have described a panel of monoclonal antibodies for schizophrenia brain studies (Brain Res 1989;500:379). Detailed results with 3 of these antibodies are now reported. Antibody EP10 binding to schizophrenia (sch) brain homogenates was increased compared to controls (con) (U=18, p=.007, n=11 sch, 11 con). This increase appeared to be globally distributed in 5 brain regions. Tissue staining with EP10 indicated a pattern typical for synaptic antigens. Immunoblotting revealed an EP10 reactive band at about 38 kd. A similar protein band immunoprecipitated by EP10 was reactive with an antibody against synaptophysin. This, or a similar synaptic molecule may be elevated in the brain in schizophrenia. Antibody EP1 binding to sch caudate was decreased compared to con (U=2, p=.01, n=7 sch, 5 con). Tissue staining showed neuronal cell bodies as well as apical and basilar dendrites. Antibody EP7 binding to temporal cortex from male cases of sch was reduced compared to male con (U=3, p=.05, n=8 sch, 4 con). In tissue sections EP7 appeared to stain distal axonal profiles. Biochemical studies on the EP1 and EP7 antigens are in progress. These preliminary results using the EP antibodies indicate that several distinct molecular abnormalities may be present in the schizophrenia brain.

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556.20

ABNORMAL GROWTH OF FIBROBLASTS FROM SCHIZOPHRENIC PATIENTS IS NOT DUE TO ACUTE NEUROLEPTIC EFFECT.

H. LAEV, R. REDDY,* S. MUKHERJEE* AND S.P. MAHADIK. Division of Neuroscience, NYSPI & Columbia Univ., N.Y., N.Y.

We have observed abnormal growth and morphology of skin fibroblasts from schizophrenic patients compared with normal controls. Cultures were established from skin biopsies of 14 schizophrenic patients and 12 age-matched normal subjects. Fibroblast cultures from these patients showed differences on the following growth parameters. Initial growth: fibroblasts from schizophrenic patients took considerably longer to establish than those from controls. Established cultures were obtained from most normals at <1 month as compared to 2-4 months for schizophrenic patients. Rate of growth: doubling time for fibroblasts from schizophrenics was markedly longer than that of normals. Morphological differences: fibroblasts from normals showed typical uniform, long, spindle-like appearance and unidirectional orientation; fibroblasts from schizophrenics exhibited random sizes (shorter, flatter) and orientation. Since all of these patients were on neuroleptic treatment, confounding effects of acute neuroleptics on these growth characteristics are difficult to resolve. To address this issue, fibroblast cultures from skin biopsies of patients (N=6) off-drug at the time of biopsy, normals (N=6) challenged in culture with haloperidol and, normal established cultures (N=4) challenged with haloperidol in culture were studied. The initial growth of fibroblasts from off-drug was improved but was not identical to normals. Rate of growth and morphology were still abnormal. There was no effect of haloperidol challenge of skin biopsy of normals or normal fibroblast culture on any of the growth parameters. Data indicate that acute haloperidol has marginal effect on initial growth only and abnormalities observed in patient fibroblasts are probably related primarily to disease process. Further studies with skin biopsies from never medicated patients will help to understand interaction of treatment with disease process.

556.21

RESPONSE LATENCY STRUCTURE IN SCHIZOPHRENIA AS RELATED TO DA OVERSTIMULATION THEORY.

M. Lyon and N. Lyon, Center for Schizophrenia Research, Department of Psychiatry, University of Arkansas for Medical Sciences, Little Rock, AR 72205.

Prior investigations have shown the presence of increased switching between responses and increasing stereotypy of responses in schizophrenic patients responding on a two-choice decisional task (Lyon et al., 1986; Lyon and Gerlach, 1988). This was interpreted as support for the effects of dopaminergic overstimulation effects on response sequencing as predicted by Lyon and Robbins (1975) and Robbins and Watson (1981). If this interpretation is correct then the response latency structure should also be more consistently patterned in schizophrenic patients, with resulting increases in number of fixed patterns and in the variety of patterns found to be repeated.

The present study examines the structure of response latency patterns between two-choice decisional responses produced by schizophrenic patients (N=17) and age, sex, and educationally matched normal control subjects (N=17) from the same sample used by Lyon et al. (1986). Sequential time range patterns in the latencies were screened by a computer program (Magnusson, M.S., Revue des Conditions de Travail, 1988) which selected only patterns which occurred at least 3 times with a variability of interresponse latencies having a probability of $P<0.0001$.

Results showed that schizophrenic patients had significantly more ($P=0.025$) total patterns than control subjects, and also a greater variety of significant ($P<0.0001$) individual response patterns. This occurred despite the fact that schizophrenic subjects had a greater number of individual "outlier" latencies that prevented some patterns from being significant. These results give further support to the twin principles of increased switching and final stage stereotypy as important markers in schizophrenic behavior.

556.22

EFFECTS OF INTRAVENTRICULAR KAINIC ACID ON SENSORY GATING OF THE RAT N40 AUDITORY EVOKED POTENTIAL.

H.T. Nagamoto, K.E. Stevens, L.L. Fuller*, S. Bernal*, R. Johnson*, and G.M. Rose Depts. of Pharmacology and Psychiatry, UCHSC, & VAMC, Denver, Colo. 80262

The N40 auditory evoked potential recorded in response to the second of a closely spaced pair of stimuli is reduced compared to the first. This gating phenomenon is attenuated in schizophrenic humans, and can be disrupted by amphetamine in humans and rats. This study evaluated the effects of intraventricular (i.c.v.) kainic acid administration on N40 gating in unanesthetized Sprague-Dawley rats. Gating mechanisms were assessed by measuring the suppression of the N40 response to a 71 dB test click stimulus following an identical conditioning stimulus given 0.5 sec earlier. In control rats, N40 showed a significant reduction to the second stimulus. Amphetamine interfered with this decrement; subsequent haloperidol administration restored normal gating. Similar results have been seen in anesthetized rats recorded from both vertex and the CA3 region of hippocampus. Ten male Sprague-Dawley rats received bilateral i.c.v. injections of 0.5-0.7 μl kainic acid. Vertex recordings were obtained 2 weeks to 4 months after infusion, and results were compared with 15 unlesioned rats. The kainic acid treated rats had significantly lower mean conditioning N40 amplitudes and higher conditioning-testing ratios. These parameters did not vary over time in either lesioned or unlesioned animals. Pyramidal cell loss in region CA3 of the lesioned rats varied between 41-88%. However, the extent of the damage to CA3 did not correlate with any N40 parameter. (Supported by P50 MH44212-01.)

556.23

DOPAMINE D1, BUT NOT D2, RECEPTOR BLOCKADE REVERSES AMPHETAMINE-INDUCED CHANGES IN AUDITORY GATING.

K.E. Stevens, L.L. Fuller* and G.M. Rose. Dept. of Pharmacology, UCHSC; and VAMC, Denver, CO 80262

The N40 auditory evoked potential recorded in response to the second of a closely spaced pair of stimuli is reduced compared to the first. This gating phenomenon is disrupted by administration of amphetamine. In addition, both conditioning and test evoked potential amplitudes are reduced compared to unmedicated trials. These changes are reversed by haloperidol. Here we report the results of selective dopamine receptor blockade on the amphetamine-induced changes. Rats were chronically implanted with a recording electrode at the vertex. Following recovery, the N40 potentials in response to paired, 71 dB click stimuli (presented 0.5 s apart, every 15 s) were assessed repeatedly over several days. Animals with normal gating were then given amphetamine (1.83 mg/kg, i.p.) to confirm drug-induced disruption of gating. Following this, animals were given either the D1 antagonist, SCH 23390 (0.5 mg/kg, i.p.), or the D2 antagonist, ±sulpiride (40 mg/kg, i.p.), and the effects on N40 observed. Blockade of D1 receptors reversed the amphetamine-induced changes in N40, yielding responses not different from the unmedicated state. In contrast, D2 receptor antagonism had no effect on amphetamine-induced alterations in N40. Administration of the antagonists alone had no effect on normal responses. Thus, changes in N40 amplitude, and the concomitant disruption of gating, induced by amphetamine appear to be mediated through a dopaminergic, D1 receptor mediated, mechanism. (Supported by P50 MH44212-01.)

556.24

PARTIAL HIPPOCAMPAL DAMAGE ABOLISHES FACILITATIVE EFFECT OF STRESS ON SIMPLE LEARNING: FURTHER ANALYSIS OF AN ANIMAL MODEL OF SCHIZOPHRENIA.

K.S. Seybold, M. Campion*, J. Kendall* and R.L. Port. Depts of Psychology, Grove City College, Grove City, PA 16127 and Slippery Rock University, Slippery Rock, PA, 16057.

A putative animal model of schizophrenia has been developed based on hippocampal neuropathy described in humans (Jeste & Lohr, 1989). Preliminary analyses (Seybold, et al, 1989) revealed that slight hippocampal damage in rats produced a facilitation of simple learning similar to the effects found in schizophrenic humans. The present experiment evaluated the effects of stress, which facilitates performance in normal subjects but may have deleterious effects on schizophrenic subjects, on simple learning. Adult hooded rats received bilateral intraventricular injections of saline (CONT), 0.5m kainic acid (EXP-L) or 1.5m kainic acid (EXP-H). After recovery, half of the animals were exposed to a cold-water swim (5° C, 3.5 min) and all were trained in shuttlebox avoidance for 30 trials on the following three days. Stress facilitated acquisition in the CONT group but not in the EXP-L or EXP-H groups.